TITLE
PEPTIDASE-CLEAVABLE, TARGETED ANTINEOPLASTIC DRUGS AND THEIR THERAPEUTIC USE

FIELD OF THE INVENTION

This invention is directed to antineoplastic agents conjugated to enzyme-cleavable peptides comprising the amino acid recognition sequence of a membrane-bound and/or cell-secreted peptidase, and to the use of such conjugated compounds as chemotherapeutic agents in the targeted treatment of cancers.

BACKGROUND OF THE INVENTION

Many anti-tumor compounds are restricted in their use because of their narrow therapeutic index, that is, the toxicities induced when the compounds are administered above certain dose levels outweigh the benefits thereby afforded. Anthracycline (*e.g.* doxorubicin) therapy, for example, is limited in that administration of the drug at levels in excess of cumulative 500 to 550 mg doxorubicin/m^2 produces a substantial risk of cardiotoxicity and myelosuppression (von Hoff, et al.). However, compounds such as doxorubicin often remain the drug of choice for particular forms of chemotherapy; therefore it would be quite useful to develop means of lowering the compounds' toxicities whilst maintaining their therapeutic potential.

One means of approaching this objective that has been tried for several decades is the design of prodrug molecules that are differentially activated in tumor tissue, that is, drug molecules inactive or significantly less active upon administration that are selectively processed in tumor tissue so as to be therapeutically active therein. Leu-Dox (the amino acid leucine conjugated to the anthracycline doxorubicin), for example, is a prodrug found to require hydrolysis of the amino acid from the prodrug by intracellular proteases in order to release the anthracycline (Boven, et al. (1990)). Conversion of Leu-Dox to Dox in mice occurs rapidly, although incompletely, to approximately 20% overall conversion (de Jong, et al. (1992a)). A similar observation has been made upon administration of Leu-Dox to humans (de Jong, et al. (1992b); Canal, et al.); in a Phase I trial, approximately 25% conversion of Leu-Dox to Dox occurred rapidly in the tumor tissue. Moreover, in a human ovarian tumor xenograft mouse model, Leu-Dox has

been shown to be a more effective anti-tumor agent than free doxorubicin, at equitoxic doses (Boven et al. (1992)).

Conjugation of additional amino acids to Leu-Dox may further decrease the availability of this compound to cells which do not secrete the requisite protease, and hence, further limits the compound's activity outside of tumors. In this regard, for example, Denmeade et al. have shown that a peptide-doxorubicin pro-drug targeted to the prostate-specific antigen ("PSA"). Ac-HSSKLQ-Leu-Dox is a substrate for the PSA protease and is active against prostate tumor cells which express the protease activity. Furthermore, other mono and dipeptide conjugates on anthracyclines in addition to Leu-Dox have also been shown to have biological activity (Masquelier, et al.; Baurain, et al.). While a comprehensive analysis of dipeptide-anthracycline conjugates has not been reported, compounds consisting of Leu-Leu-Daunorubicin, Ala-Leu-Daunorubicin, and Leu-Ala-Daunorubicin have been shown to have considerable biological activity.

Various matrix-metalloproteinases ("MMPs") have been described, and have had associated with them identifiable peptide cleavage sites (Nagase, et al.; McGeehan, et. al.). Moreover, the association between metastatic tumor progression has been made. In this regard, multiple researchers have shown that the enzymes MMP-2, MMP-9 and, more recently, MMP-14 (MT1-MMP) are associated with tumor progression (see, e.g., McDonnell and Fingleton; MacDougall and Matrisian). Increased expression of MMP-2 has also been reported in lung, stomach and breast carcinomas as compared to corresponding normal tissues. Increased expression of MMPs is not limited to the tumor itself. Increased expression of MMP-2 and MMP-14 has been observed in stromal and endothelial cells which are proximal to the tumor (e.g., Soini, Brummer). Thus, the level of MMP expressed is elevated at the tumor site.

Elevated expression of MMPs in tumor and supporting tissues implies that elevated activity is also present. While pro-forms of MMP-2 and MMP-9 enzyme are secreted by cells and readily detected in human serum and urine (Garbisa, et al.; Moses, et al.), the active form of the enzyme is found on the cell surface. In the case of MMP-2, the pro-form can be activated at the cell surface by the transmembrane enzyme, MMP-14 (Sato, et al.; Kurschatt, et al.). Activation of pro-MMP-2 has also been

described to occur through binding of the pro-form of the enzyme to an integrin (Brooks, et al.). Activation of MMP-9 has been shown to occur through specific binding to the cell surface antigen, CD-44 (Yu and Stamenkovic). Based on these findings, it is anticipated that elevated MMP protease activity will be highest on the surface of tumor cells, so differential activation of the pro-drugs will be highest at the tumor site.

Safavy et al. (A. Safavy et al. (J. Med. Chem. 42:4919-4924 (1999)) describe the attachment of a seven amino acid synthetic peptide to the antitumor agent paclitaxel.

Trouet and Baurain describe tumor-activated prodrug compounds in US Patent 5,962,216, issues Oct. 5, 1999.

WO 99/02175, WO 98/18493 and WO 98/10651 conjugate certain prostate specific antigen ("PSA") cleavable peptides to cytotoxic agents.

WO 98/16240 attaches peptides to lipids, for subsequent inclusion of the resulting conjugates in liposomes so as to target delivery of the vesicles' cytotoxic agent contents to tumors.

WO 00/33888 describes peptide conjugates of doxorubicin that are processed by an enzyme called trouase.

WO 00/21571 describes the use of FAP (Fibroblast Activation Protein) to deliver doxorubicin to tumors.

WO 00/64486 claims MMP activated conjugates for delivery of substances to tumors.

However, there remains a need to develop chemotherapeutic prodrug compounds which are inactive or significantly less active upon administration, thereby lowering the compounds' toxicities, that are selectively processed in or near tumor tissue so as to become therapeutically active anticancer agents.

The current invention discloses novel compounds useful for the treatment of cancer which comprises a matrix metalloproteinase (MMP) enzyme-cleavable peptide conjugated to doxorubicin. Furthermore, the current invention discloses novel compounds useful for the treatment of cancer which upon cleavage by a matrix metalloproteinase produces a second peptide doxorubicin substrate which can be further cleaved or processed by aminopeptidases expressed in the tumor environment. None of the references above suggest the compounds of the current invention.

SUMMARY OF THE INVENTION

This invention provides a compound comprising an enzyme-cleavable peptide conjugated to an antineoplastic agent, e.g., an anthracycline, vinca alkaloid, bleomycin, mitomycin, taxane, cytotoxic nucleotide, pteridine, or podophyllotoxin. An enzyme-cleavable peptide is a peptide comprising an amino acid sequence capable of being selectively recognized and cleaved by a membrane-bound and/or cell-secreted peptidase, for example a matrix metalloproteinase. Such compounds are useful in the treatment of cancer.

Also provided herein is a pharmaceutical composition comprising said compounds and a pharmaceutically acceptable carrier. Further provided herein is a method of delivering compounds of this invention to the cells of a mammal afflicted with a cancer, or other disorder, which comprises contacting the cells with the compound in the presence of a peptidase capable of cleaving the peptide.

It is appreciated that certain features of the invention, which are for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are for brevity, described in the context of a single embodiment, may also be provided for separately or in any suitable subcombination.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a compound comprising an antineoplastic agent conjugated to an enzyme-cleavable peptide.

In a first embodiment the invention provides a compound of Formula (I):

$$E^{cp}\text{-}A$$

(I)

or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide conjugated to A and selected from:

Cap- Paa -Xa2 -Gly - Xp1 - Laa -;

Cap- Xa2 - Gly - Xp1 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Laa -;

Cap- Gly - Xp1 - Xp2 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Paa - Xa2 - Sar - Xp1 - Laa -;

Cap- Xa2 - Sar - Xp1 - Laa -;

Cap- Paa - Xa2 - Sar - Xp1 - Xp2 - Laa -;

Cap- Xa2 - Sar - Xp1 - Xp2 - Laa -;

Cap- Sar - Xp1 - Xp2 - Laa -;

Cap- Paa - Xa2 - Sar - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Xa2 - Sar - Xp1 - Xp2 - Xp3 - Laa -; and

Cap- Sar - Xp1 - Xp2 - Xp3 - Laa -;

Paa is a Pro, Hyp, Aze, homo-Pro, Chg, Fph, Npa, Tzc, or proline mimetic;

Xa2 is an amino acid;

Xp1 is an amino acid wherein -Gly-Xp1- or -Sar-Xp1- form a bond cleavable by a matrixin;

Xp2 is an amino acid;

Xp3 is an amino acid;

Laa is an amino acid selected from Leu, Ile, Nle, β-homo-Leu, Hol, Hos, Ala, α-Ala, Cha, Cba, Cba, Cta, 4-pyridyl-Ala, 3-pyridyl-Ala, 2-pyridyl-Ala, Gly, Abu, Aib, Iva, Nva, Ahx, Aph, Amh, Phe, Bip, Glu, Arg, Trp, Tyr, O-(C_1-C_4 alkyl)-Tyr, O-(phenyl(C_1-C_4 alkyl)-)-Tyr, (C_3-C_8 alkyl)-Gly, and aminoalkyl carboxylic acid;

Cap is an N-terminus group selected from R-; Xa4-; and R-Xa4-;

Xa4- is an amino acid;

R is an amino capping group;

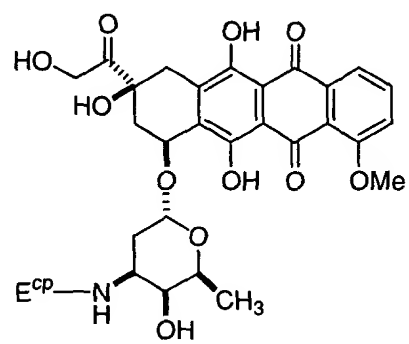
and

A is an antineoplastic agent.

[2] In a preferred embodiment the invention provides a compound of Formula (I) wherein A is doxorubicin, a doxorubicin derivative, or a doxorubicin analogue.

[3] In a more preferred embodiment the invention provides a compound of Formula (I) wherein A is doxorubicin.

[4] In a preferred embodiment the invention provides a compound of Formula (Ia):



(Ia)

or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

Cap- Paa - Xa2 - Gly - Xp1 - Laa -;

Cap- Xa2 - Gly - Xp1 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Laa -;

Cap- Gly - Xp1 - Xp2 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Paa - Xa2 - Sar - Xp1 - Laa -;

Cap- Xa2 - Sar - Xp1 - Laa -;

Cap- Paa - Xa2 - Sar - Xp1 - Xp2 - Laa -;

Cap- Xa2 - Sar - Xp1 - Xp2 - Laa -;

Cap- Sar - Xp1 - Xp2 - Laa -;

Cap- Paa - Xa2 - Sar - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Xa2 - Sar - Xp1 - Xp2 - Xp3 - Laa -; and

Cap- Sar - Xp1 - Xp2 - Xp3 - Laa -;

Paa is a Pro, Hyp, Aze, homo-Pro, Chg, Fph, Npa, Tzc, or proline mimetic;

Xa2 is an amino acid;

Xp1 is an amino acid wherein -Gly-Xp1- or -Sar-Xp1- form a bond cleavable by a matrixin;

Xp2 is an amino acid;

Xp3 is an amino acid;

Laa is an amino acid selected from Leu, Ile, Nle, β-homo-Leu, Hol, Hos, Ala, α-Ala, Cha, Cba, Cba, Cta, 4-pyridyl-Ala, 3-pyridyl-Ala, 2-pyridyl-Ala, Gly, Abu, Aib, Iva, Nva, Ahx, Aph, Amh, Phe, Bip, Glu, Arg, Trp, Tyr, O-(C_1-C_4 alkyl)-Tyr, O-(phenyl(C_1-C_4 alkyl)-)-Tyr, (C_3-C_8 alkyl)-Gly, and aminoalkyl carboxylic acid;

Cap is an N-terminus group selected from R-; Xa4-; and R-Xa4-;

Xa4- is an amino acid;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)-(CH_2)_vC(=O)$-,

wherein v is 1, 2, 3, 4, 5, or 6;

$H_3CO-(CH_2CH_2O)_t-CH_2C(=O)$-,

$HO_2CCH_2O-(CH_2CH_2O)_t-CH_2C(=O)$-,

$H_2N-(CH_2CH_2O)_t-CH_2C(=O)$-, and

$H_3CC(=O)HN-(CH_2CH_2O)_t-CH_2C(=O)$-,

wherein t is 1, 2, 3, or 4;

$R^1-C(=O)$-;

$R^1-S(=O)_2$-;

R^1-NHC(=O)-;

R^{1a}-CH_2C(=O)-;

proline substituted with -OR^3;

C_1-C_4 alkyl substituted with 0-1 R^4;

2-carboxyphenyl-C(=O)-; and

-(O=)C-phenyl-C(=O)-;

R^1 is C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from -OH, methoxy and -CO_2H;

5-6 membered heterocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH, methoxy or -CO_2H;

phenyl substituted with 0, 1, or 2 substituents selected from -OH, methoxy and -CO_2H; or

C_1-C_6 alkyl substituted with 0-4 R^{1a};

R^{1a} is -OH, C_1-C_3 alkyl, C_1-C_4 alkoxy, -CO_2H, -$N(CH_2CH_2)_2N$-R^2 , -SO_3H;

C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

5-6 membered hetereocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH;

phenyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

R^2 is -H, H_2N(C_2-C_4 alkyl)-, acetyl(H)N(C_2-C_4 alkyl)-, or acetyl;

R^3 is -H, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, phenyl, or benzyl;

R^4 is -OH, C_1-C_3 alkyl, C_1-C_4 alkoxy, -CO_2H, -$N(CH_2CH_2)_2N$-R^2 ;

C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

5-6 membered hetereocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH; or

C_6-C_{10} carbocycle substituted with 0, 1, or 2 substituents selected from methoxy and -OH.

[5] In a preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

Cap- Paa - Xa2 - Gly - Xp1 - Laa -;

Cap- Xa2 - Gly - Xp1 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Laa -;

Cap- Gly - Xp1 - Xp2 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Gly - Xp1 - Xp2 - Xp3 - Laa -;

Paa is a Pro, Hyp, Aze, homo-Pro, Chg, Fph, Npa, Tzc, or proline mimetic;

Xa2 is an amino acid;

Xp1 is an amino acid wherein -Gly-Xp1- forms a bond cleavable by a matrixin;

Xp2 is an amino acid;

Xp3 is an amino acid;

Laa is an amino acid selected from Leu, Ile, Nle, β-homo-Leu, Hol, Hos, Ala, β-Ala, Cha, Cba, Cba, Cta, 4-pyridyl-Ala, Abu, Aib, Iva, Nva, Phe, Bip, Tyr,

O-benzyl-Tyr; and

Cap is an N-terminus group selected from R-; Xa4-; and R-Xa4-;

Xa4- is an amino acid;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)-(CH_2)_vC(=O)-$,

wherein v is 1, 2, 3, or 4;

$H_3CO-(CH_2CH_2O)_t-CH_2C(=O)-$,

$HO_2CCH_2O-(CH_2CH_2O)_t-CH_2C(=O)-$,

$H_2N-(CH_2CH_2O)_t-CH_2C(=O)-$, and

$H_3CC(=O)HN-(CH_2CH_2O)_t-CH_2C(=O)-$,

wherein t is 1, 2, or 3;

$R^1-C(=O)-$;

$R^1-S(=O)_2-$;

$R^1-NHC(=O)-$;

$R^{1a}-CH_2C(=O)-$;

proline substituted with $-OR^3$;

C_1-C_4 alkyl substituted with 0-1 R^4;

$HO_3SCH_2CH(NH_2)C(=O)-$;

2-carboxyphenyl-C(=O)-; and

-(O=)C-phenyl-C(=O)-;

R^1 is C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from -OH, methoxy and $-CO_2H$;

5-6 membered heterocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH, methoxy or $-CO_2H$;

phenyl substituted with 0, 1, or 2 substituents selected from -OH, methoxy and $-CO_2H$; or

C_1-C_6 alkyl substituted with 0-4 R^{1a};

R^{1a} is -OH, C_1-C_3 alkyl, C_1-C_4 alkoxy, $-CO_2H$, $-N(CH_2CH_2)_2N-R^2$, $-SO_3H$;

C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

5-6 membered hetereocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH;

phenyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

R^2 is -H, $H_2N(C_2$-C_4 alkyl)-, acetyl(H)N(C_2-C_4 alkyl)-, or acetyl;

R^3 is -H, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, phenyl, or benzyl;

R^4 is -OH, C_1-C_3 alkyl, C_1-C_4 alkoxy, $-CO_2H$, $-N(CH_2CH_2)_2N-R^2$;

C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

5-6 membered hetereocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH; or

C_6-C_{10} carbocycle substituted with 0, 1, or 2 substituents selected from methoxy and -OH.

[6] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by the matrixin selected from MMP-2, MMP-9, and MMP-14.

[7] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by the matrixin selected from MMP-2 and MMP-9.

[8] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by the matrixin MMP-14.

[9] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by MMP-2 , MMP-9, and MMP-14.

[10] In a preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

Cap- Paa - Xa2 - Gly - Xp1 - Laa -;

Cap- Xa2 - Gly - Xp1 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Laa -;

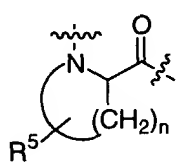
Cap- Gly - Xp1 - Xp2 - Laa -;

Cap- Paa - Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Xa2 - Gly - Xp1 - Xp2 - Xp3 - Laa -;

Cap- Gly - Xp1 - Xp2 - Xp3 - Laa -;

wherein -Gly-Xp1- forms a bond cleavable by a matrixin;

Paa is a Pro, Hyp, Aze, homo-Pro, Chg, Fph, Npa, Tzc, or proline mimetic of formula:  ; wherein R^5 is selected from H, halogen, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy, and benzyloxy; and n is 2, 3, 4, or 5;

Xa2 is an amino acid selected from

Hof, Leu, His, Arg, Gln, Ile, Val, Lys, (R)-Leu, Orn, β-Ala, γ-Abu, Cha, Chg, Dap, Cit, N-methyl-Leu, valerolactam, N,N-dimethyl-Lys, 4-aza-Phe, morpholinylpropyl-Gly, N-methylpiperazinepropyl-Gly, 4-aza-Hof, Ala, Asn, Asp, Aze, Cys, Glu, Gly, Hyp, Irg, Met, Phe, Phe(4-fluoro), Pro, Sar, Ser, Thr, Trp, Tyr, Cya, Hca, and Spa;

Xp1 is an amino acid selected from Hof; Leu; Bip; Phe; nor-Leu; Tha; Phg; Val; Glu; Asn; Ser; Ala; homo-Tyr; Aze; 4-aza-Hof; O-(3-pyridyl)-Tyr; O-(4-pyridyl)-Tyr; O-benzyl-Tyr; O-benzyl-Thr; O-benzyl-Ser; O-methyl-Ser; O-allyl-Ser; 4-nitro-Hof; N-methyl-Leu;

O-(4-pyridylmethyl)-Tyr; 4-hydroxy-phenyl-Gly; phenylpropyl-Gly; styryl-Ala , or 2Nal;

Xp2 is an amino acid selected from Tyr; Ala; Ser; Leu; Gln; Val; Glu, His; Lys; Arg; Orn; Aze; Hof; homo-Tyr; Cit; 4-aza-Phe; N,N-Dimethyl-Lys; Dab; Dap; Asn, Asp, Aze, Cha, Cys, Gly, Hyp, Ile, Irg, Met, Phe, Phe(4-fluoro), Pro, Sar, Thr, Trp, Cya, Hca, Spa, morpholinylpropyl-Gly; O-(4-pyridylmethyl)-Tyr; and N-methylpiperazinepropyl-Gly;

Xp3 is an amino acid selected from Tyr, Ala, Ser, Leu, Hof, Arg, Asn, Asp, Aze, Cha, Cys, Dpa, Gln, Glu, Gly, His, Hyp, Ile, Irg, Lys, Met, Orn, Phe, Phe(4-fluoro), Pro, Sar, Thr, Trp, and Val;

Laa is an amino acid selected from Leu, Ile, Nle, β-homo-Leu, Hol, Hos, Ala, β-Ala, Cha, Cba, Cba, Cta, 4-pyridyl-Ala, Abu, Aib, Iva, Nva, and Phe;

Cap is an N-terminus group selected from R-; Xa4-; and R-Xa4-;

Xa4- is an amino acid selected from Gly, Pro, γ-Glu, Dmg, Ala, Arg, Asn, Asp, β-Asp, Aze, Cha, Cys, Dpa, Gln, Glu, His, Hyp, Ile, Irg, Leu, Lys, Met, Orn, Phe, Sar, Ser, Thr, Trp, Tyr, or Val;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2CH_2C(=O)$-;

$H_3COCH_2CH_2OCH_2C(=O)$-,

$H_3COCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$HO_2CCH_2OCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$H_3CC(=O)HNCH_2CH_2OCH_2C(=O)$-,

$H_3CC(=O)HNCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2N(CH_2CH_2)_2NCH_2C(O)$-;

$H_3CC(=O)HNCH_2CH_2N(CH_2CH_2)_2NCH_2C(O)$-;

$H_3CC(=O)N(CH_2CH_2)_2NCH_2C(O)$-;

$O(CH_2CH_2)_2NCH_2CH_2NHC(O)$-

$HO_2CCH_2C(CO_2H)(OH)CH_2C(=O)$-,

$HO_2CCH_2C(CH_3)(OH)CH_2C(=O)$-,

2-carboxycyclohexyl-C(=O)-;

2-carboxycyclopentyl-C(=O)-;

carbobenzyloxy;

4-methoxy-benzenesulfonyl;

cyclopropylcarbonyl;

cyclobutylcarbonyl;

3-pyridinecarbonyl;

2-pyrazinecarbonyl;

tetrazoleacetyl;

pivaloyl;

methoxyacetyl;

hydroxyproline; and

4-(2-(5,6,7,8-tetrahydronaphthenyl))butyl.

[11] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by the matrixin selected from MMP-2, MMP-9, and MMP-14.

[12] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by the matrixin selected from MMP-2 and MMP-9.

[13] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by the matrixin MMP-14.

[14] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Xp1- forms a bond cleavable by MMP-2 , MMP-9, and MMP-14.

[15] In a preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

Cap- Paa - Xa2 - Gly - Leu - Laa -;
Cap- Paa - Xa2 - Gly - Hof - Laa -;
Cap- Xa2 - Gly - Leu - Laa -;
Cap- Xa2 - Gly - Hof - Laa -;
Cap- Paa - Xa2 - Gly - Leu - Xp2 - Laa -;
Cap- Paa - Xa2 - Gly - Hof - Xp2 - Laa -;
Cap- Xa2 - Gly - Leu - Xp2 - Laa -;
Cap- Xa2 - Gly - Hof - Xp2 - Laa -;
Cap- Gly - Leu - Xp2 - Laa -; and
Cap- Gly - Hof - Xp2 - Laa -;

wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by a matrixin;

Paa is a Pro, Hyp, Aze, homo-Pro, or Npa;

Xa2 is an amino acid selected from
Hof, Leu, His, Arg, Gln, Ile, Val, Lys, (R)-Leu, Orn, β-Ala, γ-Abu, Cha, Chg, Dap, Cit, N-methyl-Leu, valerolactam, N,N-dimethyl-Lys, 4-aza-Phe, morpholinylpropyl-Gly, N-methylpiperazinepropyl-Gly, 4-aza-Hof, Ala, Asn, Asp, Aze, Cys, Glu, Gly, Hyp, Irg, Met, Phe, Phe(4-fluoro), Pro, Sar, Ser, Thr, Trp, Tyr, Cya, Hca, and Spa;

Xp2 is an amino acid selected from Tyr; Ala; Ser; Leu; Gln; Val; Glu, His; Lys; Arg; Orn; Aze; Hof; homo-Tyr; Cit; 4-aza-Phe; N,N-Dimethyl-Lys; Dab; Dap; Asn, Asp, Aze, Cha, Cys, Gly, Hyp, Ile, Irg, Met, Phe,

Phe(4-fluoro), Pro, Sar, Thr, Trp, Cya, Hca, Spa, morpholinylpropyl-Gly; O-(4-pyridylmethyl)-Tyr; and N-methylpiperazinepropyl-Gly;

Laa is an amino acid selected from Leu, Cha, Nle, and Hol;

Cap is an N-terminus group selected from R-; Xa4-; and R-Xa4-;

Xa4- is an amino acid selected from Gly, Pro, γ-Glu, and Dmg;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2CH_2C(=O)$-;

$H_3COCH_2CH_2OCH_2C(=O)$-,

$H_3COCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$HO_2CCH_2OCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$H_3CC(=O)HNCH_2CH_2OCH_2C(=O)$-,

$H_3CC(=O)HNCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2N(CH_2CH_2)_2NCH_2C(O)$-;

$H_3CC(=O)HNCH_2CH_2N(CH_2CH_2)_2NCH_2C(O)$-;

$H_3CC(=O)N(CH_2CH_2)_2NCH_2C(O)$-;

$O(CH_2CH_2)_2NCH_2CH_2NHC(O)$-

$HO_2CCH_2C(CO_2H)(OH)CH_2C(=O)$-,

$HO_2CCH_2C(CH_3)(OH)CH_2C(=O)$-,

2-carboxycyclohexyl-C(=O)-;

2-carboxycyclopentyl-C(=O)-;

carbobenzyloxy;

4-methoxy-benzenesulfonyl;

cyclopropylcarbonyl;

cyclobutylcarbonyl;

3-pyridinecarbonyl;

2-pyrazinecarbonyl;

tetrazoleacetyl;

pivaloyl;

methoxyacetyl;

hydroxyproline; and

4-(2-(5,6,7,8-tetrahydronaphthenyl))butyl.

[16] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin selected from MMP-2, MMP-9, and MMP-14.

[17] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin selected from MMP-2 and MMP-9.

[18] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin MMP-14.

[19] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by MMP-2 , MMP-9, and MMP-14.

[14] In a preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

Cap- Paa - Xa2 - Gly - Leu - Leu -;

Cap- Paa - Xa2 - Gly - Leu - Cha -;

Cap- Paa - Xa2 - Gly - Leu - Nle -;

Cap- Paa - Xa2 - Gly - Leu - Hol -;

Cap- Paa - Xa2 - Gly - Hof - Leu -;

Cap- Paa - Xa2 - Gly - Hof - Cha -;

Cap- Paa - Xa2 - Gly - Hof - Nle -;

Cap- Paa - Xa2 - Gly - Hof - Hol -;

Cap- Paa - Xa2 - Gly - Leu - Xp2 - Leu -;

Cap- Paa - Xa2 - Gly - Leu - Xp2 - Cha -;

Cap- Paa - Xa2 - Gly - Leu - Xp2 - Nle -;

Cap- Paa - Xa2 - Gly - Leu - Xp2 - Hol -;

Cap- Paa - Xa2 - Gly - Hof - Xp2 - Leu -;

Cap- Paa - Xa2 - Gly - Hof - Xp2 - Cha -;

Cap- Paa - Xa2 - Gly - Hof - Xp2 - Nle -;

Cap- Paa - Xa2 - Gly - Hof - Xp2 - Hol -;

wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by a matrixin;

Paa is a Pro, Hyp, Aze, homo-Pro, or Npa;

Xa2 is an amino acid selected from Hof, Leu, His, Arg, Gln, Ile, Val, Lys, (R)-Leu, Orn, β-Ala, γ-Abu, Cha, Chg, Dap, Cit, N-methyl-Leu, valerolactam, N,N-dimethyl-Lys, 4-aza-Phe, morpholinylpropyl-Gly, N-methylpiperazinepropyl-Gly, 4-aza-Hof, Ala, Asn, Asp, Aze, Cys, Glu, Gly, Hyp, Irg, Met, Phe, Phe(4-fluoro), Pro, Sar, Ser, Thr, Trp, and Tyr;

Xp2 is an amino acid selected from Tyr; Ala; Ser; Leu; Gln; Val; Glu, His; Lys; Arg; Orn; Aze; Hof; homo-Tyr; Cit; 4-aza-Phe; N,N-Dimethyl-Lys; Dab; Dap; Asn, Asp, Aze, Cha, Cys, Gly, Hyp, Ile, Irg, Met, Phe, Phe(4-fluoro), Pro, Sar, Thr, Trp; morpholinylpropyl-Gly; O-(4-pyridylmethyl)-Tyr; and N-methylpiperazinepropyl-Gly;

Cap is an N-terminus group selected from R-; Xa4-; and R-Xa4-;

Xa4- is an amino acid selected from Gly, Pro, γ-Glu, and Dmg;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2C(=O)$-;
$HOC(=O)CH_2CH_2CH_2CH_2C(=O)$-;
$H_3COCH_2CH_2OCH_2C(=O)$-;
$H_3COCH_2CH_2OCH_2CH_2OCH_2C(=O)$-;
2-carboxycyclohexyl-C(=O)-;
2-carboxycyclopentyl-C(=O)-; and
tetrazoleacetyl.

[21] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin selected from MMP-2, MMP-9, and MMP-14.

[22] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin selected from MMP-2 and MMP-9.

[23] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin MMP-14.

[24] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by MMP-2 , MMP-9, and MMP-14.

[25] In a preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

Cap- Xa2 - Gly - Leu - Leu -;
Cap- Xa2 - Gly - Leu - Cha -;
Cap- Xa2 - Gly - Leu - Nle -;
Cap- Xa2 - Gly - Leu - Hol -;
Cap- Xa2 - Gly - Hof - Leu -;
Cap- Xa2 - Gly - Hof - Cha -;

Cap- Xa2 - Gly - Hof - Nle -;

Cap- Xa2 - Gly - Hof - Hol -;

Cap- Xa2 - Gly - Leu - Xp2 - Leu -;

Cap- Xa2 - Gly - Leu - Xp2 - Cha -;

Cap- Xa2 - Gly - Leu - Xp2 - Nle -;

Cap- Xa2 - Gly - Leu - Xp2 - Hol -;

Cap- Xa2 - Gly - Hof - Xp2 - Leu -;

Cap- Xa2 - Gly - Hof - Xp2 - Cha -;

Cap- Xa2 - Gly - Hof - Xp2 - Nle -; and

Cap- Xa2 - Gly - Hof - Xp2 - Hol -;

wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by a matrixin;

Xa2 is an amino acid selected from

Hof, Leu, His, Arg, Gln, Ile, Val, Lys, (R)-Leu, Orn, β-Ala, γ-Abu, Cha, Chg, Dap, Cit, N-methyl-Leu, valerolactam, N,N-dimethyl-Lys, 4-aza-Phe, morpholinylpropyl-Gly, N-methylpiperazinepropyl-Gly, 4-aza-Hof, Ala, Asn, Asp, Aze, Cys, Glu, Gly, Hyp, Irg, Met, Phe, Phe(4-fluoro), Pro, Sar, Ser, Thr, Trp, and Tyr;

Xp2 is an amino acid selected from Tyr; Ala; Ser; Leu; Gln; Val; Glu, His; Lys; Arg; Orn; Aze; Hof; homo-Tyr; Cit; 4-aza-Phe; N,N-Dimethyl-Lys; Dab; Dap; Asn, Asp, Aze, Cha, Cys, Gly, Hyp, Ile, Irg, Met, Phe, Phe(4-fluoro), Pro, Sar, Thr, Trp; morpholinylpropyl-Gly; O-(4-pyridylmethyl)-Tyr; and N-methylpiperazinepropyl-Gly;

Cap is an N-terminus group selected from R-; Xa4-; and R-Xa4-;

Xa4- is an amino acid selected from Gly, Pro, γ-Glu, and Dmg;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2CH_2C(=O)$-;

$H_3COCH_2CH_2OCH_2C(=O)$-;

$H_3COCH_2CH_2OCH_2CH_2OCH_2C(=O)$-;

2-carboxycyclohexyl-C(=O)-;

2-carboxycyclopentyl-C(=O)-; and

tetrazoleacetyl.

[26] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin selected from MMP-2, MMP-9, and MMP-14.

[27] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin selected from MMP-2 and MMP-9.

[28] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by the matrixin MMP-14.

[29] In a preferred embodiment the invention provides a compound of Formula (Ia), wherein -Gly-Leu- and -Gly-Hof- form a bond cleavable by MMP-2 , MMP-9, and MMP-14.

[30] In another preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

SEQ. ID. NO: 185: R-γ-E -P-Orn-G-Hof-E-L-;

SEQ. ID. NO: 186: R-γ-E -P-L-G-(O-benzyl-S)-Y-L-;

SEQ. ID. NO: 187: R -γ-E -P-L-G-(O-benzyl-S)-Y-Nle-;

SEQ. ID. NO: 188: R -P-L-G-(O-benzyl-S)-Y-L-;

SEQ. ID. NO: 189: R -P-L-G-(O-methyl-S)-Y-L-;

SEQ. ID. NO: 190: R -P-L-G-(azaHof)-Y-L-;

SEQ. ID. NO: 191: R -P-L-G-Hof-Y-L-;

SEQ. ID. NO: 192: R -P-L-G-Hof-E-L-;

SEQ. ID. NO: 193: R -P-L-G-(O-benzyl-S)-Y-Nle-;

SEQ. ID. NO: 194: R -P-L-G-(O-methyl-S)-Y- Nle -;

SEQ. ID. NO: 195: R -P-L-G-(azaHof)-Y- Nle -;

SEQ. ID. NO: 196: R -P-L-G-Hof-Y- Nle -;

SEQ. ID. NO: 197: R -P-L-G-Hof-E- Nle -;

SEQ. ID. NO: 198: R -P-L-G-(O-benzyl-S)-Y-Hol-;

SEQ. ID. NO: 199: R -P-L-G-(O-methyl-S)-Y- Hol -;

SEQ. ID. NO: 200: R -P-L-G-(azaHof)-Y- Hol -;

SEQ. ID. NO: 201: R -P-L-G-Hof-Y- Hol -;

and

SEQ. ID. NO: 202: R -P-L-G-Hof-E- Hol -;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)-(CH_2)_vC(=O)$-,

wherein v is 1, 2, 3, 4, 5, or 6;

$H_3CO-(CH_2CH_2O)_t-CH_2C(=O)$-,

$HO_2CCH_2O-(CH_2CH_2O)_t-CH_2C(=O)$-,

$H_2N-(CH_2CH_2O)_t-CH_2C(=O)$-, and

$H_3CC(=O)HN-(CH_2CH_2O)_t-CH_2C(=O)$-,

wherein t is 1, 2, 3, or 4;

R^1-C(=O)-;

R^1-S(=O)$_2$-;

R^1-NHC(=O)-;

R^{1a}-$CH_2C(=O)$-;

proline substituted with -OR^3;

C_1-C_4 alkyl substituted with 0-1 R^4;

2-carboxyphenyl-C(=O)-; and

-(O=)C-phenyl-C(=O)-;

R^1 is C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from -OH, methoxy and $-CO_2H$;

5-6 membered heterocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH, methoxy or $-CO_2H$;

phenyl substituted with 0, 1, or 2 substituents selected from -OH, methoxy and $-CO_2H$; or

C_1-C_6 alkyl substituted with 0-4 R^{1a};

R^{1a} is -OH, C_1-C_3 alkyl, C_1-C_4 alkoxy, $-CO_2H$, $-N(CH_2CH_2)_2N-R^2$, $-SO_3H$;

C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

5-6 membered hetereocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH;

phenyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

R^2 is -H, $H_2N(C_2$-C_4 alkyl)-, acetyl(H)N(C_2-C_4 alkyl)-, or acetyl;

R^3 is -H, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, phenyl, or benzyl;

R^4 is -OH, C_1-C_3 alkyl, C_1-C_4 alkoxy, $-CO_2H$, $-N(CH_2CH_2)_2N-R^2$;

C_3-C_6 cycloalkyl substituted with 0, 1, or 2 substituents selected from methoxy and -OH;

5-6 membered hetereocycle; said heterocycle being saturated, partially saturated or unsaturated; said heterocycle containing 1, 2, 3, or 4 heteroatoms selected from N, O, and S; said heterocycle optionally substituted with 1 or 2 -OH; or

C_6-C_{10} carbocycle substituted with 0, 1, or 2 substituents selected from methoxy and -OH.

[31] In a preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

SEQ. ID. NO: 185: R-γ-E -P-Orn-G-Hof-E-L-;

SEQ. ID. NO: 186: R-γ-E -P-L-G-(O-benzyl-S)-Y-L-;

SEQ. ID. NO: 187: R -γ-E -P-L-G-(O-benzyl-S)-Y-Nle-;

SEQ. ID. NO: 188: R -P-L-G-(O-benzyl-S)-Y-L-;

SEQ. ID. NO: 189: R -P-L-G-(O-methyl-S)-Y-L-;

SEQ. ID. NO: 190: R -P-L-G-(azaHof)-Y-L-;

SEQ. ID. NO: 191: R -P-L-G-Hof-Y-L-;

SEQ. ID. NO: 192: R -P-L-G-Hof-E-L-;

SEQ. ID. NO: 193: R -P-L-G-(O-benzyl-S)-Y-Nle-;

SEQ. ID. NO: 194: R -P-L-G-(O-methyl-S)-Y- Nle -;

SEQ. ID. NO: 195: R -P-L-G-(azaHof)-Y- Nle -;

SEQ. ID. NO: 196: R -P-L-G-Hof-Y- Nle -;

SEQ. ID. NO: 197: R -P-L-G-Hof-E- Nle -;

SEQ. ID. NO: 198: R -P-L-G-(O-benzyl-S)-Y-Hol-;

SEQ. ID. NO: 199: R -P-L-G-(O-methyl-S)-Y- Hol -;

SEQ. ID. NO: 200: R -P-L-G-(azaHof)-Y- Hol -;

SEQ. ID. NO: 201: R -P-L-G-Hof-Y- Hol -;

and

SEQ. ID. NO: 202: R -P-L-G-Hof-E- Hol -;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2CH_2C(=O)$-;

$H_3COCH_2CH_2OCH_2C(=O)$-,

$H_3COCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$HO_2CCH_2OCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$H_3CC(=O)HNCH_2CH_2OCH_2C(=O)$-,

$H_3CC(=O)HNCH_2CH_2OCH_2CH_2OCH_2C(=O)$-,

$H_2NCH_2CH_2N(CH_2CH_2)_2NCH_2C(O)$-;

$H_3CC(=O)HNCH_2CH_2N(CH_2CH_2)_2NCH_2C(O)$-;

$H_3CC(=O)N(CH_2CH_2)_2NCH_2C(O)$-;

$O(CH_2CH_2)_2NCH_2CH_2NHC(O)$-

$HO_2CCH_2C(CO_2H)(OH)CH_2C(=O)$-,

$HO_2CCH_2C(CH_3)(OH)CH_2C(=O)$-,

2-carboxycyclohexyl-C(=O)-;

2-carboxycyclopentyl-C(=O)-;

carbobenzyloxy;

4-methoxy-benzenesulfonyl;

cyclopropylcarbonyl;

cyclobutylcarbonyl;

3-pyridinecarbonyl;

2-pyrazinecarbonyl;

tetrazoleacetyl;

pivaloyl;

methoxyacetyl;

hydroxyproline; and

4-(2-(5,6,7,8-tetrahydronaphthenyl))butyl.

[32] In a preferred embodiment the invention provides a compound of Formula (Ia), or a pharmaceutically acceptable salt form thereof, wherein;

E^{cp} is an enzyme cleavable peptide selected from:

SEQ. ID. NO: 185: R-γ-E -P-Orn-G-Hof-E-L-;

SEQ. ID. NO: 186: R-γ-E -P-L-G-(O-benzyl-S)-Y-L-;

SEQ. ID. NO: 187: R -γ-E -P-L-G-(O-benzyl-S)-Y-Nle-;

SEQ. ID. NO: 188: R -P-L-G-(O-benzyl-S)-Y-L-;

SEQ. ID. NO: 189:	R -P-L-G-(O-methyl-S)-Y-L-;
SEQ. ID. NO: 190:	R -P-L-G-(azaHof)-Y-L-;
SEQ. ID. NO: 191:	R -P-L-G-Hof-Y-L-;
SEQ. ID. NO: 192:	R -P-L-G-Hof-E-L-;
SEQ. ID. NO: 193:	R -P-L-G-(O-benzyl-S)-Y-Nle-;
SEQ. ID. NO: 194:	R -P-L-G-(O-methyl-S)-Y- Nle -;
SEQ. ID. NO: 195:	R -P-L-G-(azaHof)-Y- Nle -;
SEQ. ID. NO: 196:	R -P-L-G-Hof-Y- Nle -;
SEQ. ID. NO: 197:	R -P-L-G-Hof-E- Nle -;
SEQ. ID. NO: 198:	R -P-L-G-(O-benzyl-S)-Y-Hol-;
SEQ. ID. NO: 199:	R -P-L-G-(O-methyl-S)-Y- Hol -;
SEQ. ID. NO: 200:	R -P-L-G-(azaHof)-Y- Hol -;
SEQ. ID. NO: 201:	R -P-L-G-Hof-Y- Hol -;
and	
SEQ. ID. NO: 202:	R -P-L-G-Hof-E- Hol -;

R is selected from: $H_3CC(=O)$-;

$HOC(=O)CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2C(=O)$-;

$HOC(=O)CH_2CH_2CH_2CH_2C(=O)$-;

$H_3COCH_2CH_2OCH_2C(=O)$-;

$H_3COCH_2CH_2OCH_2CH_2OCH_2C(=O)$-; and

tetrazoleacetyl.

[33] In another preferred embodiment the invention provides a compound selected from:

SEQ.ID.NO: 1:	4-methoxy-benzenesulfonyl- β -Ala-G-Hof-Y-L-Dox;
SEQ.ID.NO: 2:	1,2-C_6H_4 $(CO)_2$-H-G-Hof-Y-L-Dox;
SEQ.ID.NO: 3:	acetyl -P-L-G-L-L-Dox;
SEQ.ID.NO: 4:	acetyl -P-(R)L-G-L-L-Dox;
SEQ.ID.NO: 5:	acetyl -P -(β -Ala) -G-L-L-Dox;
SEQ.ID.NO: 6:	acetyl -P -(γ-Abu) -G-L-L-Dox;
SEQ.ID.NO: 7:	acetyl -P-Cha-G-L-L-Dox;

SEQ.ID.NO: 8:	P-L-G-L-L-Dox;
SEQ.ID.NO: 9:	$\mathrm{MeOCH_2CH_2OCH_2C(=O)}$- P-L-G-L-L-Dox;
SEQ.ID.NO: 10:	$\mathrm{MeOCH_2CH_2OCH_2CH_2OCH_2C(=O)}$- P-L-G-L-L-Dox;
SEQ.ID.NO: 11:	$\mathrm{H_2NCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)}$- P-L-G-L-L-Dox;
SEQ.ID.NO: 12:	$\mathrm{AcHNCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)}$- P-L-G-L-L-Dox;
SEQ.ID.NO: 13:	$\mathrm{AcN(CH_2CH_2)_2NCH_2C(=O)}$- P-L-G-L-L-Dox;
SEQ.ID.NO: 17:	Dmg- P-R-Sar-Hof-L-Dox;
SEQ.ID.NO: 18:	acetyl-P-H-G-Hof-L-Dox;
SEQ.ID.NO: 19:	acetyl-P-Orn-G-Hof-L-Dox;
SEQ.ID.NO: 20:	acetyl-P-Dap-G-Hof-L-Dox;
SEQ.ID.NO: 21:	acetyl-P-Cit-G-Hof-L-Dox;
SEQ.ID.NO: 22:	acetyl-P-L-G-(O-(3-pyridyl-))Y-L-Dox;
SEQ.ID.NO: 23:	acetyl-P-L-G-(O-(4-pyridyl-))Y-L-Dox;
SEQ.ID.NO: 24:	acetyl-P-L-G-(4-aza-)Hof-L-Dox;
SEQ.ID.NO: 25:	acetyl-P-L-G-(O-benzyl-)S-L-Dox;
SEQ.ID.NO: 26:	Cbz-P-L-G-(O-(4-pyridylmethyl-))Y-L-Dox;
SEQ.ID.NO: 27:	acetyl -P-L-Sar-L-L-Dox;
SEQ.ID.NO: 28:	acetyl -P- (N-Me-)L-G-L-L-Dox;
SEQ.ID.NO: 29:	acetyl -P- L-G-(N-Me-)L-L-Dox;
SEQ.ID.NO: 30:	acetyl -Hyp- L-G-L-L-Dox;
SEQ.ID.NO: 31:	acetyl -Tzc- L-G-L-L-Dox;
SEQ.ID.NO: 32:	acetyl -(Homo-P)-L-G-L-L-Dox;
SEQ.ID.NO: 33:	acetyl -(Homo-P)-L-G- Hof -L-Dox;
SEQ.ID.NO: 34:	acetyl -(Homo-P)-Orn-G- Hof -L-Dox;
SEQ.ID.NO: 35:	acetyl -Nipecotate -L-G-L-L-Dox;
SEQ.ID.NO: 36:	acetyl -Aze-L-G-L-L-Dox;
SEQ.ID.NO: 37:	acetyl -Chg -L-G-L-L-Dox;
SEQ.ID.NO: 38:	acetyl -P-valerolactam -G-L-L-Dox;
SEQ.ID.NO: 41:	acetyl -L-G-L-Y-L-Dox;
SEQ.ID.NO: 42:	cyclopropylcarbonyl -L-G-L-Y-L-Dox;
SEQ.ID.NO: 43:	cyclobutylcarbonyl -L-G-L-Y-L-Dox;
SEQ.ID.NO: 44:	pivaloyl -L-G-L-Y-L-Dox.
SEQ.ID.NO: 45:	Hyp-G-P-L-G-L-L-Dox;
SEQ.ID.NO: 46:	acetyl -P-L-G-L-A-L-Dox;
SEQ.ID.NO: 47:	acetyl -P-L-G-L-Y-L-Dox;
SEQ.ID.NO: 48:	Peg -P-L-G-L-Y-L-Dox;
SEQ.ID.NO: 49:	$\mathrm{H_3CC(=O)NH}$-Peg -P-L-G-L-Y-L-Dox;
SEQ.ID.NO: 50:	$\mathrm{AcHNCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)}$- P-L-G-L-Y-L-Dox;
SEQ.ID.NO: 51:	acetyl -P-L-G-L-S-L-Dox;
SEQ.ID.NO: 52:	acetyl-G-P-L-G-L-L-Dox;
SEQ.ID.NO: 53:	$\mathrm{O(CH_2CH_2)NCH_2CH_2NHC(=O)}$-G-P-L-G-L-L-Dox;
SEQ.ID.NO: 55:	acetyl -P-L-G-L-L-L-Dox;
SEQ.ID.NO: 58:	Cbz-G-P-L-G-L-L-Dox;
SEQ.ID.NO: 59:	$\mathrm{AcHNCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)}$-G-P-L-G-L-L-Dox;
SEQ.ID.NO: 60:	$\mathrm{H_2NCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)}$-G-P-L-G-L-L-Dox;
SEQ.ID.NO: 61:	Dmg-P-L-G-L-L-Dox;
SEQ.ID.NO: 62:	acetyl- γ-E -P-L-G-L-L-Dox;
SEQ.ID.NO: 65:	methoxyacetyl-G-P-L-G-L-L-Dox;

SEQ.ID.NO: 66:	Dmg-P-L-G-Tha-L-Dox;
SEQ.ID.NO: 67:	Dmg-P-L-G-Phg-L-Dox;
SEQ.ID.NO: 68:	Dmg-P-L-G-(O-benzyl-Y)-L-Dox;
SEQ.ID.NO: 69:	Dmg-P-L-G-Bip-L-Dox;
SEQ.ID.NO: 77:	acetyl-G-P-Q-G-L-L-Dox;
SEQ.ID.NO: 78:	acetyl-G-P-R-G-L-L-Dox;
SEQ.ID.NO: 82:	acetyl-G-P-L-G-V-L-Dox;
SEQ.ID.NO: 83:	acetyl-G-P-L-G-Hof-L-Dox;
SEQ.ID.NO: 84:	acetyl-G-P-L-A-L-L-Dox;
SEQ.ID.NO: 85:	Dmg-P-I-G-Bip-L-Dox;
SEQ.ID.NO: 86:	Dmg-P-Chg-G-Bip-L-Dox;
SEQ.ID.NO: 87:	acetyl-G-P-V-G-L-L-Dox;
SEQ.ID.NO: 88:	Dmg-P-I-G-L-L-Dox;
SEQ.ID.NO: 89:	Dmg-P-R-G-Bip-L-Dox;
SEQ.ID.NO: 91:	acetyl-G-P-L-G-E-L-Dox;
SEQ.ID.NO: 92:	Dmg-P-K-G-Bip-L-Dox;
SEQ.ID.NO: 95:	Dmg -P-R-Sar-Hof-R-L-Dox;
SEQ.ID.NO: 96:	Dmg -P-R-G-Hof-R-L-Dox;
SEQ.ID.NO: 97:	Dmg -P-R-G-Bip-R-L-Dox;
SEQ.ID.NO: 98:	acetyl-G-P-L-G-N-L-Dox;
SEQ.ID.NO: 99:	acetyl-G-P-L-G-S-L-Dox;
SEQ.ID.NO: 100:	acetyl-G-P-L-G-(4-hydroxy-phenyl-G)-L-Dox;
SEQ.ID.NO: 101:	acetyl -P-L-G-Hof-H-L-Dox;
SEQ.ID.NO: 102:	acetyl -P-L-G-Hof-A-L-Dox;
SEQ.ID.NO: 103:	acetyl -P-L-G-Hof-Y-L-Dox;
SEQ.ID.NO: 104:	acetyl -P-L-G-Hof- (morpholinylpropyl-G) -L-Dox;
SEQ.ID.NO: 105:	acetyl -γ-E -P-L-G-Hof-Y-L-Dox;
SEQ.ID.NO: 106:	succinyl -P-L-G-Hof-Y-L-Dox;
SEQ.ID.NO: 107:	acetyl -P-L-G-Hof- (O-(4-pyridylmethyl)-Y)-L-Dox;
SEQ.ID.NO: 108:	acetyl -P-L-G-(homo-Y)-Y-L-Dox;
SEQ.ID.NO: 109:	acetyl -P-L-G-(4-aza-Hof)-Y-L-Dox;
SEQ.ID.NO: 110:	acetyl -P-L-G-(O-(4-pyridyl-)-Y)-Y-L-Dox;
SEQ.ID.NO: 111:	acetyl -P-L-G- (phenylpropyl-G) -Y-L-Dox;
SEQ.ID.NO: 112:	acetyl -P-L-G-(styryl-A)-Y-L-Dox;
SEQ.ID.NO: 113:	acetyl -P-L-G-(O-benzyl-S)-Y-L-Dox;
SEQ.ID.NO: 114:	acetyl -P- (N,N-dimethyl-K)-G-Hof-Y-L-Dox;
SEQ.ID.NO: 115:	acetyl -P-L-G-Hof-Dap-L-Dox;
SEQ.ID.NO: 116:	acetyl -P-L-G-Hof-Orn-L-Dox;
SEQ.ID.NO: 117:	Peg -P-L-G-Hof-Orn-L-Dox;
SEQ.ID.NO: 118:	acetyl -γ-E -P-L-G-Hof-Orn-L-Dox;
SEQ.ID.NO: 119:	γ-E -P-L-G-Hof-Orn-L-Dox;
SEQ.ID.NO: 120:	acetyl -P-Orn-G-Hof-Orn-L-Dox;
SEQ.ID.NO: 121:	acetyl -P-Orn-G-Hof-Y-L-Dox;
SEQ.ID.NO: 122:	acetyl -γ-E -P-Orn-G-Hof-E-L-Dox;
SEQ.ID.NO: 123:	acetyl -P-Orn-G-L-Y-L-Dox;
SEQ.ID.NO: 124:	acetyl -P-(4-aza-F)-G-L-Y-L-Dox;
SEQ.ID.NO: 125:	acetyl -P-L-G-Hof-Dab-L-Dox;
SEQ.ID.NO: 126:	acetyl -P-L-G-Hof-K-L-Dox;

SEQ.ID.NO: 127:	acetyl -P-L-G-Hof- (N,N-dimethyl-K)-L-Dox;
SEQ.ID.NO: 128:	Dmg -P-L-G-Hof-(N,N-dimethyl-K)-L-Dox;
SEQ.ID.NO: 129:	Peg -P-L-G-Hof- (N,N-dimethyl-K)-L-Dox;
SEQ.ID.NO: 130:	acetyl -γ-E -P-L-G-Hof-(N,N-dimethyl-K)-L-Dox;
SEQ.ID.NO: 131:	γ-E -P-L-G-Hof-(N,N-dimethyl-K)-L-Dox;
SEQ.ID.NO: 132:	acetyl -P-L-G-Hof- (N,N-dimethyl-K)-Nle-Dox;
SEQ.ID.NO: 133:	acetyl -P-L-G-Hof- (N,N-dimethyl-K)-Cha-Dox;
SEQ.ID.NO: 134:	acetyl -P-L-G-Hof-Cit-L-Dox;
SEQ.ID.NO: 135:	acetyl -γ-E -P-L-G-Hof-Cit-L-Dox;
SEQ.ID.NO: 136:	acetyl -P-L-G-Hof-Q-L-Dox;
SEQ.ID.NO: 137:	acetyl -P-L-G-Hof-(4-aza-F)-L-Dox;
SEQ.ID.NO: 138:	acetyl -P-L-G-Hof-V-L-Dox;
SEQ.ID.NO: 139:	acetyl -γ-E -P-L-G-Hof-E-L-Dox;
SEQ.ID.NO: 140:	acetyl-G-Aze-L-G-L-L-Dox;
SEQ.ID.NO: 141:	acetyl -(4-fluoro-F)- L-G-L-L-Dox;
SEQ.ID.NO: 142:	acetyl -(homo-P)-L-G-L-Y-L-Dox;
SEQ.ID.NO: 143:	acetyl -(homo-P)-L-G-Hof-Orn-L-Dox;
SEQ.ID.NO: 144:	acetyl -Aze-L-G-L-Y-L-Dox;
SEQ.ID.NO: 145:	acetyl -Aze-L-G-Hof-Orn-L-Dox;
SEQ.ID.NO: 154:	acetyl -P-L-G-L-L-A-L-Dox;
SEQ.ID.NO: 155:	acetyl -P-L-G-L-Y-A-L-Dox;
SEQ.ID.NO: 156:	acetyl -G -P-L-G-L-A-L-Dox;
SEQ.ID.NO: 157:	acetyl -P-L-G-L-A-A-L-Dox;
SEQ.ID.NO: 158:	acetyl -P-L-G-L-A-L-L-Dox;
SEQ.ID.NO: 159:	acetyl -P-L-G-L-L-S-L-Dox;
SEQ.ID.NO: 160:	acetyl -P-L-G-L-L-L-L-Dox;
SEQ.ID.NO: 161:	Dmg -P-L-G-L-Y-L-Dox;
SEQ.ID.NO: 162:	Dmg -P-R-G-Phg-Y-L-Dox;
SEQ.ID.NO: 163:	acetyl -G -P-L-G-L-R-L-Dox;
SEQ.ID.NO: 164:	4-(2-(5,6,7,8-tetrahydronaphthenyl))butyl -G-Hof-Y-L-Dox;
SEQ.ID.NO: 165:	acetyl -P-L-G-Hof-(N-methylpiperazinepropyl-G)-L-Dox;
SEQ.ID.NO: 166:	tetrazoleacetyl -P-L-G-Hof-Y-L-Dox;
SEQ.ID.NO: 167:	tetrazoleacetyl -P-L-G-(O-benzyl-S)-Y-L-Dox;
SEQ.ID.NO: 168:	tetrazoleacetyl -P-L-G-Hof-Y-Nle-Dox;
SEQ.ID.NO: 169:	P-L-G-(O-benzyl-S)-Y-L-Dox;
SEQ.ID.NO: 170:	acetyl -P-L-G-Hof-(homoY)-L-Dox;
SEQ.ID.NO: 171:	acetyl -P-AzaHof-G-AzaHof-Y-L-Dox;
SEQ.ID.NO: 172:	acetyl -P-L-G-(O-allyl-S)-Y-L-Dox;
SEQ.ID.NO: 173:	acetyl -P-L-G-(4-nitro-Hof)-Y-L-Dox;
SEQ.ID.NO: 174:	acetyl -P-L-G-Hof-AzaHof-L-Dox;
SEQ.ID.NO: 175:	acetyl -P-L-G-(O-methyl-S)-Y-L-Dox;
SEQ.ID.NO: 176:	acetyl -γ-E -P-L-G-(O-benzyl-S)-Y-L-Dox;
SEQ.ID.NO: 177:	acetyl -γ-E -P-L-G-(O-benzyl-S)-Y-Nle-Dox;
SEQ.ID.NO: 178:	3-pyridinecarbonyl -P-L-G-Hof-Y-L-Dox;
SEQ.ID.NO: 179:	2-pyrazinecarbonyl -P-L-G-Hof-Y-L-Dox;
SEQ.ID.NO: 180:	acetyl -P-L-G-Hof- (N,N-dimethyl-K)-Nle-Dox;
SEQ.ID.NO: 182:	acetyl -P-L-G-Hof-Y-Hol-Dox;

SEQ.ID.NO: 183: acetyl -P-L-G-Thr(O-Benzyl)-Y-L-Dox;
SEQ.ID.NO: 184: acetyl -γ-E -P-L-G-Hof-Y-Nle-Dox;

[34] In another preferred embodiment the invention provides a compound selected from:

SEQ.ID.NO: 39: acetyl -G-P-L-G-L-F-Dox;
SEQ.ID.NO: 40: acetyl -G-P-L-G-F-F-Dox;
SEQ.ID.NO: 54: acetyl-G-P-L-G-L-Y-Dox;
SEQ.ID.NO: 56: acetyl-G-P-L-G-Bip-F-Dox;
SEQ.ID.NO: 57: acetyl-G-P-L-G-Nle-F-Dox;
SEQ.ID.NO: 63: acetyl-G-P-L-G-Tha-F-Dox;
SEQ.ID.NO: 64: acetyl-G-P-L-G-Phg-F-Dox;
SEQ.ID.NO: 70: acetyl-G-P-L-G-F-Bip-Dox;
SEQ.ID.NO: 71: acetyl-G-P-L-G-L-Bip-Dox;
SEQ.ID.NO: 72: acetyl-G-P-L-G-(2Nal)-Bip-Dox;
SEQ.ID.NO: 73: acetyl-G-P-L-G-F-A-Dox;
SEQ.ID.NO: 74: acetyl-G-P-L-G-Bip-A-Dox;
SEQ.ID.NO: 75: acetyl-G-P-L-G-L-A-Dox;
SEQ.ID.NO: 76: acetyl-G-P-L-G-(O-benzyl-Y)-F-Dox;
SEQ.ID.NO: 79: acetyl-G-P-L-G-L-(4-pyridyl-A)-Dox;
SEQ.ID.NO: 80: acetyl-G-P-L-G-L-R-Dox;
SEQ.ID.NO: 81: acetyl-G-P-L-G-L-W-Dox;
SEQ.ID.NO: 90: acetyl-G-P-L-G-L-(O-benzyl-Y)-Dox;
SEQ.ID.NO: 93: acetyl-G-P-L-G-L-E-Dox;
SEQ.ID.NO: 94: acetyl-G-P-L-G-Bip-E-Dox;
SEQ.ID.NO: 146: acetyl -P-L-G-L-Y-G-Dox;
SEQ.ID.NO: 147: acetyl -P-L-G-Hof-Y-G-Dox;
SEQ.ID.NO: 148: acetyl -P-L-G-L-Y-(β-homo-L)-Dox;
SEQ.ID.NO: 149: acetyl -P-L-G-Hof-Y-(β-homo-L)-Dox;
SEQ.ID.NO: 150: acetyl -P-L-G-L-Y- (β-Ala)-Dox;
SEQ.ID.NO: 151: acetyl -P-L-G-L-Y-Ahx -Dox;
SEQ.ID.NO: 152: acetyl -P-L-G-L-Y-Aph -Dox;
SEQ.ID.NO: 153: acetyl -P-L-G-L-Y-Amh -Dox;
SEQ.ID.NO: 181: acetyl -P-L-G-Hof-Y-Hos-Dox;

[35] In second embodiment the invention provides a pharmaceutical composition comprising a compound of Formula (I) or (Ia) and a pharmaceutically acceptable carrier.

[36] In third embodiment the invention provides for a method of treating a mammal afflicted with a cancer comprising adminstering to a mammal afflicted with a cancer a therapeutically effective amount of a compound of Formula (I) or (Ia).

[37] In a preferred embodiment the invention provides for a method of treating a mammal afflicted with a cancer wherein the cancer is a breast, ovarian, brain, stomach, lung, colon, prostate or liver cancer or wherein the cancer is a leukemia, lymphoma, carcinoma, sarcoma, or melanoma.

[38] In fourth embodiment the invention provides for a method of delivering a compound to the cells of a mammal afflicted with a cancer comprising contacting the cells of a mammal afflicted with a cancer with a of Formula (I) or (Ia), wherein the contacting is in the presence of a peptidase comprising a matrixin.

[39] In a preferred embodiment the invention provides for a method wherein the cancer is a breast, ovarian, brain, stomach, lung, colon, prostate or liver cancer or wherein the cancer is a leukemia, lymphoma, carcinoma, sarcoma, or melanoma.

In a fifth embodiment the invention provides for a compound of Formula (I):

$$E^{cp}\text{-}A \tag{I}$$

comprising an enzyme-cleavable peptide, E^{cp}, conjugated to an antineoplastic agent, A.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the antineoplastic agent is an anthracycline, vinca alkaloid, bleomycin, mitomycin, taxane, cytotoxic nucleotide, pteridine or podophyllotoxin.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the antineoplastic agent is an anthracycline.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the antineoplastic agent is the anthracycline doxorubicin.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the amino acid sequence is selected from the group consisting of PLGL,

PLGLL, PLGLAL, PLGLYL, PLGLYAL, PLGLAAL, PLGLLSL, PLGLLAL, PLGLLYL, GPLGL, GPLGLL, PLGHof, PLG-(O-Benzyl)-S, PLGHofYL, PLG-(O-Benzyl)-SYL, PLGHofEL, and GPLGLAL.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the amino acid sequence is selected from the group consisting of PLGL, PLGLL, PLGLAL, PLGLYL, PLGLLAL, PLGLLYL, GPLGL, GPLGLL and GPLGLAL.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the enzyme-cleavable peptide comprises an amino acid sequence recognized by a peptidase wherein the peptidase is a matrixin.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the peptidase is a matrixin comprising MMP-2, MMP-9, or MMP-14.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the agent is doxorubicin and wherein the enzyme-cleavable peptide comprises an amino acid sequence selected from the group consisting of PLGL, PLGLL, PLGLAL, PLGLYL, PLGLLAL, PLGLLYL, PLGLYAL, GPLGL, GPLGLL and GPLGLAL.

In a preferred embodiment the invention provides for a compound of Formula (I) wherein the agent is doxorubicin and wherein the enzyme-cleavable peptide comprises an amino acid sequence recognized by a peptidase selected from the group consisting of matrixin MMP-2 , MMP-9, or MMP-14.

In another preferred embodiment the invention provides for a pharmaceutical composition comprising the compound of Formula (I) and a pharmaceutically acceptable carrier.

In another preferred embodiment the invention provides method of delivering a compound to the cells of a mammal afflicted with a cancer comprising contacting the cells of a mammal afflicted with a cancer with the compound of Formula (I), wherein the contacting is in the presence of a peptidase comprising a matrixin.

In another preferred embodiment the invention provides a method of delivering a compound of Formula (I) to the cells of a mammal afflicted with a cancer wherein the cancer is a breast, ovarian, brain, stomach, lung, colon, prostate or liver cancer or wherein the cancer is a leukemia, lymphoma, carcinoma, sarcoma, or melanoma.

In another preferred embodiment the invention provides a method of delivering a compound of Formula (I) to the cells of a mammal afflicted with a cancer wherein the anticancer agent is an anthracycline, vinca alkaloid, bleomycin, mitomycin, taxane, cytotoxic nucleotide, pteridine or podophyllotoxin.

In another preferred embodiment the invention provides a method of delivering a compound of Formula (I) to the cells of a mammal afflicted with a cancer wherein the anticancer agent is an anthracycline, vinca alkaloid, bleomycin, mitomycin, taxane, cytotoxic nucleotide, pteridine or podophyllotoxin and wherein the agent is the anthracycline doxorubicin.

Also included in the present invention are compounds as set forth above wherein the enzyme-cleavable peptide is selectively recognized by a matrixin comprising MMP-2, MMP-9, and/or MMP-14 and not selectively recognized by the enzyme human fibroblast activation protein (FAPα).

Also included in the present invention are compounds as set forth above wherein the amino acid Laa is not proline or a proline analogue wherein the substituents on the alpha nitrogen and substituents on the alpha carbon form a cyclic group.

Also included in the present invention are compounds as set forth above provided that the amino capping group, Cap, is not a polyhydroxyalkanoyl, that is, wherein the hydroxyalkanoyl capping groups are limited to those with one hydroxy substituent on the alkanoyl group.

Also included in the present invention are compounds as set forth above wherein the enzyme-cleavable peptide is selectively recognized by a matrixin comprising MMP-2, MMP-9, and/or MMP-14 and not selectively recognized by the enzyme Trouase.

Also included in the present invention are compounds as set forth above provided that the amino acid Xa2 is a natural amino acid.

Also included in the present invention are compounds as set forth above provided that Cap is not a non-natural amino acid or succinyl.

Also included in the present invention are compounds as set forth above wherein the enzyme-cleavable peptide is selectively recognized by a matrixin comprising MMP-2, MMP-9, and/or MMP-14 and not selectively recognized by prostate specific antigen (PSA).

Also included in the present invention are compounds as set forth above provided E^{cp} does not comprise a dipeptide linkage selected from -Tyr-Ser-; -Tyr-Thr-; -Phe-Ser-; -Gln-Ser-; -Gln-Thr-, and -Asn-Ser .

Also included in the present invention are compounds as set forth above provided E^{cp} is not -Gly-Gly-Arg-Leu-.

Also included in the present invention are compounds as set forth above provided E^{cp} is not -Gly-Val-Phe-Arg-.

Also included in the present invention are compounds as set forth above provided E^{cp} is not -Ala-Pro-Gly-Leu-.

Also included in the present invention are compounds as set forth above provided E^{cp} is not 2-thienylalanine-Gly-Ala-Leu-.

Also included in the present invention are compounds as set forth above provided E^{cp} is not 2-naphthylalanine -Gly-Ala-Leu-.

Also included in the present invention are compounds as set forth above provided E^{cp} is not -Gly-Leu-Gly-Leu-.

"Antineoplastic agents" as used herein means agents which have cytotoxic effects on tumor cells; these include both compounds such as alkylating agents, tubulin-binding agents, and antiproliferative agents, as well as proteins, e.g., tumor necrosis factor, interferons and various growth factors, which may negatively impact upon the

growth of cancerous cells. Specific "antineoplastic agents" suitable for use herein include, without limitation: anthracyclines, bleomycin, vinca alkaloids (e.g., vincristine and vinblastine), mitomycin, cytotoxic nucleotides, taxanes (e.g., paclitaxel and taxotere, (see DeGroot)), pteridines, podophyllotoxins, and folic acid derivatives (see Lu). Such compounds may be modified, e.g., to enhance the compounds' potential therapeutic efficacies or to ease their conjugation to peptides, at various points on their structures, by means well known to ordinarily skilled artisans.

As used herein the "antineoplastic agents" which are anthracyclines are intended to include doxorubicin, doxorubicin derivatives, and doxorubicin anologues, examples of which include, but are not limited to, doxorubicin (adriamycin), daunorubicin (daunomycin), epirubicin, detorubicin, idarubicin, esorubicin, and carminomycin, as well as, mitoxantrone. A preferred anthracycline is doxorubicin, referred to herein as "Dox" or "dox".

Enzyme cleavable peptides comprise amino acid sequences recognized and cleaved by membrane bound and/or cell-secreted peptidases, which are peptide-cleaving enzymes well known in the art to recognize particular amino acid sequences and to cleave said sequences between specific amino acids (see, e.g., Ames and Quigley et al.; Knauper et al., McGeehan et al., Nagase et al., Nakajima et al., Odake et al.). Such enzymes include, for example and without limitation, matrix metalloproteinases or "MMP's" (also refered to herein as matrixins), e.g., MMP-2, MMP-9, MMP-14, serine proteases, cysteine proteases, elastase, stromelysins, human collagenases, cathepsins, granzymes, dipeptidyl peptidases, plasmins, plasminogen activators, lysozymes and e.g., aminopeptidase P, aminopeptidase A, and aminopeptidase N. Peptides with suitable MMP substrate selectivity suitable for conjugation to cytotoxic agents herein include, for example and without limitation, those having the amino acid sequences: PLGL, PLGLL, PLGLAL, PLGLYL, PLGLLAL, PLGLALL, PLGLLLL, PLGLLYL, PLGLYAL, PLGLAAL, PLGLLSL, GPLGL, GPLGLY, GPLGLL, GPLGLAL, DPLGL, PEQGL, PQGL, and PLGL-Dpa-AR and similar sequence (Nagase). Each of these amino acid sequences optionally includes any of the various modified amino acids, e.g., hydroxyproline, described herein, and each of the sequences is optionally modified by any of the amino or carboxy terminal modifications, e.g., acetyl, described herein. Thus, in addition to the specific amino acid sequences set forth, this invention

also provides corresponding versions containing one or more natural, modified, or unnatural amino acids and one or more terminal modifications, e.g., this invention provides peptides comprising the amino acid sequence PLGLYL, as well as Hyp-PLGLYL, AcPLGLYL and AcHypPLGLYL.

As used herein "matrixin" is intended to generically describe matrix metalloproteinases or MMP's as a class of enzymes which recognize the enzyme-cleavable peptides of the compounds of the present invention. Preferred MMP's are MMP-2, MMP-9, and/or MMP-14. Matrixin does not describe the enzyme neprilysin.

As used herein "a bond cleavable by a matrixin" is intended to describe an amide bond of the enzyme-cleavable peptide which is amenable to proteolytic cleavage *in vitro* by a matrixin, as defined herein. It is intended that matrixins, as defined herein, are preferably selective for the bond cleavable by a matrixin. It is also understood that proteolytic degradation of the enzyme-cleavable peptide may occur at any bond on the enzyme-cleavable peptide following the administration of the compound *in vivo*.

Enzyme cleavable peptides must contain the minimum number of amino acids, substitutions or modifications thereof, for recognition and cleavage by the corresponding peptidase (e.g., PLGL, AA). Alternatively, the peptides' amino acid sequences may comprise one or more amino acids in addition to those minimally necessary for peptidase-mediated cleavage (e.g., peptides comprising, in order, the amino acids P, L, G and L may have the amino acid sequence PLGLL, and peptides comprising the amino acid sequence AA may actually have the sequence AAPV). Such additional amino acids are included in the peptides, at the amino and/or carboxy terminal ends, for a variety of reasons well known to ordinarily skilled artisans given the teachings of this invention, e.g., to further decrease the availability to nonpeptidase-secreting cells of compounds to which the peptides are conjugated. Additionally, the amino acid sequence remaining on the cytotoxic agent after the initiating peptidase cleavage event must be composed of sequences that are capable of being removed or processed by cellular aminopeptidases after tumor associated peptidase cleavage. (e.g., LL-Dox or LAL-Dox)

Compounds of the present invention conjugated to enzyme cleavable peptides recognized and cleaved by matrix metalloproteinases MMP-2, MMP-9, and/or MMP-14, are believed to recognize particular amino acid sequences and to cleave said

sequences containing glycine or sarcocine at the cleavage site. As such, enzyme cleavable peptides of the present invention contain the dipeptides -Gly-Xp1- or -Sar-Xp1- at the cleavage site wherein Xp1 is an amino acid which forms a bond to Gly or Sar cleavable by a free matrixin or matrix metalloproteinase. Preferred examples of Xp1 include, but are not limited to, Leu, Hof, azaHof, Ser(Omethyl), and Ser(Obenzyl). In addition to the above dipeptides, MMP-2, MMP-9, and/or MMP-14, are believed to recognize and cleave amino acid sequences -Paa-Xaa-Gly-Xp1- and -Paa-Xaa-Sar-Xp1-, wherein Paa is a proline, proline derivative, or proline mimetic and Xaa is an amino acid. Preferred examples of Paa include, but are not limited to, Pro and Hyp.

In addition to the matrix metalloproteinases (MMP's) MMP-2, MMP-9, and MMP-14 disclosed above, the present invention intends for the use of matrixins MMP-13 and MMP-8 to also be used in a cytotoxic peptide conjugate targeting approach. Enzyme/amino acid recognition sequence pairings include, for example, MMP-13 recognizing the sequence PLGL (see, e.g., Knauper et al.), and MMP-8 recognizing the sequences AAPF or AAPM; particularly where these have been N-terminal modified by succinyl or methoxysuccinyl (see, e.g., Nakajima et al). The contents of these descriptions are incorporated herein by reference.

Such peptides, as well as other enzyme-cleavable peptides, including peptides containing substitute, modified, unnatural or natural amino acids in their sequences, as well as peptides modified at their amino or carboxy terminus, are made from their component amino acids by a variety of methods well known to ordinarily skilled artisans, and practiced thereby using readily available materials and equipment, (see, e.g., The Practice of Peptide Synthesis (2nd. ed.), M. Bodanskzy and A. Bodanskzy, Springer-Verlag, New York, NY (1994), the contents of which are incorporated herein by reference). These include, for example and without limitation: solid-phase synthesis using the Fmoc protocol (see, e.g., Change and Meieinhofer, Int. J. Pept. Protein Res. 11:246-9 (1978)). Other documents describing peptide synthesis include, for example and without limitation: Miklos Bodansky, Peptide Chemistry, A Practical Textbook 1988, Springer-Verlag, N.Y; Peptide Synthesis Protocols, Michael W. Pennington and Ben M. Dunn editors, 1994, Humana Press Totowa, N.J.

As descibed hereinabove, enzyme-cleavable peptides comprise an amino acid sequence which serves as the recognition site for a peptidase capable of cleaving the peptide. The amino acids comprising the enzyme cleavable peptides may include natural, modified, or unnatural amino acids, wherein the natural, modified, or unnatural amino acids may be in either D or L configuration. Natural amino acids include the amino acids alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparganine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, and tyrosine. Natural amino acids, as used herein, have the following abbreviations:

1-Letter Code	3-Letter Code	Name
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Asparticacid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
U	Scy	Selenocysteine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

Enzyme-cleavable peptides may also comprise a variety of unnatural or modified amino acids suitable for substitution into the enzyme-cleavable peptide of the invention. A definite list of unnatural amino acids is disclosed in Roberts and Vellaccio, The Peptides, Vol. 5, 341-449 (1983) Academic Press, New York, and is incorporated herein by reference for that purpose. Examples of unnatural or modified amino acids used herein include, without limitation:

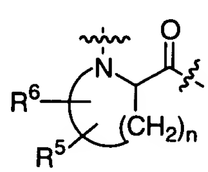
3-Letter Code	Name
Aaa	alpha-amino acid
Aad	2-aminoadipic acid (2-aminohexanedioic acid)
Aan	alpha-asparagine
Abu	2-aminobutanoic acid or 2-aminobutyric acid
γAbu	4-aminobutyric acid
Aca	2-aminocapric acid (2-aminodecanoic acid)
Acp	6-aminocaproic acid
Agn	alpha-glutamine
Ahe	2-aminoheptanoic acid
Ahx	6-aminohexanoic acid
Aib	alpha-aminoisobutyric acid (2-aminoalanine)
3-Aib	3-aminoisobutyric acid
β-Ala	beta-alanine
aHyl	allo-hydroxylysine
alle	allo-isoleucine
Amh	4-amino-7-methylheptanoic acid
Aph	4-amino-5-phenylpentanoic acid
Apm	2-aminopimelic acid (2-aminoheptanedioic acid)
App	gamma-amino-beta-hydroxybenzenepentanoic acid
Asu	2-aminosuberic acid (2-aminooctanedioic acid)
Aze	2-carboxyazetidine
Bal	beta-alanine
Bas	beta-aspartic acid
Bip	Biphenylalanine
Bly	3,6-diaminohexanoic acid (beta-lysine)
Bua	butanoic acid
Bux	4-amino-3-hydroxybutanoic acid
Cap	gamma-amino-beta-hydroxycyclohexanepentanoic acid)
Cba	cyclobutyl alanine
Cha	Cyclohexylalanine
Chg	Cyclohexylglycine
Cit	N5-aminocarbonylornithine
Cpa	cyclopentyl alanine
Cta	cyclopropyl alanine
Cya	3-sulfoalanine or cysteic acid
Dab	2,4-diaminobutanoic acid
Dap	diaminopropionic acid
Dbu	2,4-diaminobutyric acid
Dpa	diphenyl alanine
Dmg	N,N-dimethylglycine
Dpm	diaminopimelic acid
Dpr	2,3-diaminopropanoic acid or 2,3-diaminopropionic acid
Edc	S-ethylthiocysteine
EtAsn	N-ethylasparagine
EtGly	N-ethylglycine
Faf	4-aza-phenylalanine

Fph	4-fluoro-phenylalanine
Ggu	gamma-glutamic acid or (γ-E) or (γ-Glu)
Gla	gamma-carboxyglutamic acid
Glc	hydroxyacetic acid (glycolic acid)
Glp	pyroglutamic acid
Har	homoarginine
Hca	homocysteic acid
Hcy	homocysteine
Hhs	homohistidine
Hiv	2-hydroxyisovaleric acid
Hof	homophenylalanine
Hol	homoleucine or homo-L
Hop	homoproline or homo-P
Hos	homoserine
Hse	homoserine
Hva	2-hydroxypentanoic acid
Hyl	5-hydroxylysine
Hyp	4-hydroxyproline
Inc	2-carboxyoctahydroindole
Iqc	3-carboxyisoquinoline
Iva	isovaline
Lac	2-hydroxypropanoic acid (lactic acid)
Maa	mercaptoacetic acid
Mba	mercaptobutanoic acid
MeGly	N-methylglycine or sarcosine
Mhp	4-methyl-3-hydroxyproline
Mpa	mercaptopropanoic acid
Nle	norleucine
Npa	nipecotic acid
Nty	nortyrosine
Nva	norvaline
Oaa	omega-amino acid
Orn	ornithine
Pen	penicillamine (3-mercaptovaline)
Phg	2-phenylglycine
Pip	2-carboxypiperidine
Sar	sarcosine (N-methylglycine)
Spa	2-amino-3-(4-sulfophenyl)propionic acid
Spg	1-amino-1-carboxycyclopentane
Sta	statin (4-amino-3-hydroxy-6-methylheptanoic acid)
Tha	3-thienylalanine
Tml	epsilon-N-trimethyllysine
Tza	3-thiazolylalanine
Tzc	thiazolidine 4-carboxylic acid
Und	undefined
Xaa	any amino acid
Wil	alpha-amino-2,4-dioxopyrimidinepropanoic acid
2Nal	2-naphthylalanine

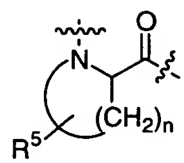
Enzyme-cleavable peptides may also comprise a variety of modified amino acids wherein an amine or hydroxy function of the amino acid has been chemically modified with an alkyl group, an alkenyl group, a phenyl group, a phenylalkyl group, a heterocyclic group, a heterocyclicalkyl group, a carbocyclic group, or a carbocyclicalkyl group. Examples of chemical modification substituents include, but are not limited to, methyl, ethyl, propyl, butyl, allyl, phenyl, benzyl, pyridyl, pyridylmethyl, and imidazolyl. "The Peptides" Vol 3, 3-88 (1981) discloses numerous suitable sidechain functional groups for modifying amino acids, and is herein incorporated for that purpose. Examples of modified amino acids include, but are not limited to, N-methylated amino acids, N-methylglycine, N-ethylglycine, N-ethylasparagine, N,N-dimethyllysine, N'-(2-imidazolyl)lysine, O-methyltyrosine, O-benzyltyrosine, O-pyridyltyrosine, O-pyridylmethyltyrosine, O-methylserine, O-t-butylserine, O-allylserine, O-benzylserine, O-methylthreonine, O-t-butylthreonine, O-benzylthreonine, O-methylaspartic acid, O-t-butylaspartic acid, O-benzylaspartic acid, O-methylglutamic acid, O-t-butylglutamic acid, and O-benzylglutamic acid,

Enzyme-cleavable peptides may also comprise a modified amino acid which is 4-azahydroxyphenylalanine (4-azaHof or azaHof), 4-aminomethylalanine, 4-pryidylalanine, 4-azaphenylalanine, morpholinylpropyl glycine, piperazinylpropyl glycine, N-methylpiperazinylpropyl glycine, 4-nitro-hydroxyphenylalanine, 4-hydroxyphenyl glycine, or a 2-(4,6-dimethylpyrimidinyl)lysine.

Enzyme-cleavable peptides may also comprise an amino acid designated Paa, which is the natural amino acid proline or can be a modified or unnatural amino acid which mimics proline. "Proline mimetics", as used herein, are of the general formula



wherein R^5 is selected from H, halogen, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy, hydroxymethyl-, phenoxy, and benzyloxy; R^6 is selected from H, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy; and n is 2, 3, 4, or 5. Preferred proline mimetics are of the general formula


; wherein R^5 is selected from H, halogen, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy, and benzyloxy; and n is 2, 3, 4, or 5. More preferred n is 3 or 4. Examples of proline mimetics are 4-hydroxyproline, 3-methylproline, 4-methylproline, 5-methylproline, 4,4-dimethylproline, 4-fluoroproline, 4,4-difluoroproline, 4-bromoproline, 4-chloroproline, 4-hydroxymethylproline, 3-hydroxyproline, 3-hydroxy-5-methylproline, 3,4-dihydroxyproline, 3-phenoxyproline, 2-azetidinecarboxylic acid, 4-methyl-2-azetidinecarboxylic acid, pipecolic acid, 5-hydroxypipecolic acid, and 4,5-dihydroxypipecolic acid. Preferred examples of proline mimetics are 4-hydroxyproline, 2-azetidinecarboxylic acid, and pipecolic acid. Examples of Paa include, but are not limited to Pro, 4-hydroxyproline, dihydroxyproline, 2-carboxyazetidine, homo-Pro, cyclohexylglycine, 4-fluoro-phenylalanine, nipecotic acid, and thiazolidine 4-carboxylic acid.

Enzyme-cleavable peptides have amino acid sequences wherein one or more of the amino acids is optionally substituted by homologous or isoteric amino acids, such that the peptides recognition and cleavage by cell-secreted peptides is not adversely affected. For example, and without limitation, the following amino acid substitutions may be made (in either direction): A - G; R - K - Orn; N - Q; D - E; I - V - L - M - Nle; F - W - Y; and S - T.

Moreover, enzyme cleavable peptides are optionally modified at the end not conjugated to the antineoplastic agent by what is known in the art as a capping group; for example, the N-terminus of the enzyme cleavable peptide is modified with a N-terminus capping group or an "amino capping group". Such modifications are for a number of reasons; for example, to increase plasma stability of the peptide against enzymatic degradation by non selective enzymes in the plasma or to increase solubility.

Amino capping groups are known in the art and occur in a variety of ways, for example, various acyl, thioacyl, alkyl, sulfonyl, phosphoryl, and phosphinyl groups comprised of 1 to 20 carbon atoms; wherein substituents on these groups may be either alkyl, aryl, alkylaryl, and so forth, which may contain the heteroatoms, O, S, and N as a substituent or in-chain component. A number of amino capping groups are recognized

by those skilled in the art of peptide synthesis. Gross and Meinhoffer, eds., The Peptides, Vol 3; 3-88 (1981), Academic Press, New York, and Greene and Wuts Protective Groups in Organic Synthesis, 315-405 (1991), J. Wiley and Sons, Inc., New York disclose numerous suitable amine protecting groups useful for the invention herein and they are incorporated herein by reference for that purpose.

In addition to the above, more preferred "amino capping groups" may be alkanoyls, hydroxylated alkanoyls, polyhydroxylated alkanoyls, aroyls, hydroxylated aroyls, polyhydroxylated aroyls, cycloalkyloyls, heterocycloyls, polyethyleneglycols, glycosylates, sugars, carboxy sugars, amino acids, dicarboxylic acids, and crown ethers; each linked to the N-terminal end of the peptide by way of an amide linkage. Examples of amino capping groups include, but are not limited to, acetyl (Ac), pivaloyl, methoxyacetyl, malonyl, succinyl (Suc), glutaryl, benzoyl, methoxy-succinyl (MeO-Suc), pyridinecarbonyl, pyrazinecarbonyl, benzyloxycarbonyl (Cbz), and t-butoxycarbonyl. Alternatively, amino capping groups containing an amine function, such as various carboxy sugars and amino acids containing basic amines; can be linked to the N-terminus of the peptide conjugate by a urea linkage.

Polyethyleneglycols as a class of compounds known as amino capping groups are ethyleneoxy compounds of general formula $H_3CO\text{-}(CH_2CH_2O)_t\text{-}CH_2C(=O)\text{-}$, wherein t is 1 to 10. Preferred polyethyleneglycols are where t is 1, 2, 3, or 4; more preferred is where t is 1 or 2. Unless otherwise specified, "polyethyleneglycol" or "PEG" or "Peg" means an amino capping group of formula $H_3COCH_2CH_2OCH_2CH_2OCH_2C(=O)\text{-}$. Polyethyleneglycols as amino capping groups can be modified to include amino-polyethyleneglycols of formula $H_2N\text{-}(CH_2CH_2O)_t\text{-}CH_2C(=O)\text{-}$, wherein t is 1, 2, 3, or 4, as well as acetamido-polyethyleneglycols of formula $H_3CC(=O)HN\text{-}(CH_2CH_2O)_t\text{-}CH_2C(=O)\text{-}$, wherein t is 1, 2, 3, or 4; as well as carboxymethyl-polyethyleneglycols of formula $HO_2CCH_2O(CH_2CH_2O)_t\text{-}CH_2C(=O)\text{-}$, wherein t is 1, 2, 3, or 4.

Moreover, an amino capping group may optionally be an amino acid modified by an alkanoyl, a dicarboxylic acid, a tricarboxylic acid, or a dicarboxylic acid ester. Examples include, but are not limited to, an acetyl (Ac), methoxyacetyl, malonyl, succinyl (Suc), glutaryl, 3-hydroxy-3-methylglutaryl (HMG), citryl, methoxy-succinyl (MeO-Suc), methoxy-malonyl, or methoxy-glutaryl group modified on the amino

terminus of, for example, serine or gamma-glutamic acid. For example, acetyl-serine (Ac-Ser), methoxysuccinyl-serine (MeO-Suc-Ser), and succinyl-serine (Suc-Ser).

Peptides are conjugated to antineoplastic agents so as to derive the compounds provided herein; said conjugation may be via either the amino or carboxy terminus of the peptide. "Conjugation," as used herein, means the linking of a peptide to a bioactive agent. Such linkage can be directly, through covalent bonding between the peptide and the agent, by means, and using reagents, well known to ordinarily skilled artisans. Covalent bonding between the peptide and agent includes the formation of an amide bond between a free amino group on the antineoplastic agent and the carboxyl group at the peptides C-terminus, or between the peptide's N-terminal amino group and a carboxyl group on the agent. Additionally, ester linkages can be formed between the C-terminal carboxyl group of the peptide and a free hydroxyl group on the antineoplastic agent or *vice versa*.

Alternatively, the peptide and antineoplastic agent can be conjugated indirectly through a linker group having free, active moities available for separate interactions with both the peptide and the agent. Such linkers include, for example, and without limitation, biscarbonyl alkyl diradicals, having a group available to form an amide bond with a free amino group on the antineoplastic agent as well as a second free group available to form an amide bond with the N-terminal amino group of the peptide. Suitable linker groups also include diaminoalkyl diradicals, having free amino groups available for amide bond formation with both the peptide's C-terminal carboxyl group and a free carboxyl group on the agent. Means of forming such amide, ester and other linkages between peptides and cytotoxic agents, either directly, or via linker groups, are well known to those of ordinary skill in the art.

Preferably, the antineoplastic agent used herein is doxorubicin and the enzyme cleavable peptide comprises an amino acid sequence recognized and cleaved by a matrixin, e.g., MMP-2, MMP-9, or MMP-14. More preferably, the peptide comprises the amino acid sequence PLGL, and can include the sequences PLGL, preferably PLGL, PLGLL, PLGLAL, PLGLYL, PLGLYAL, PLGLAAL, PLGLLSL, PLGLLAL, PLGLLYL, GPLGL, GPLGLL, PLGHof, PLG-(O-Benzyl)-S, or GPLGLAL and other sequences as exemplified in the Tables of Examples.

As used herein, "alkyl" or “alkylene” is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms; for example, "C_1-C_6 alkyl" denotes alkyl having 1 to 6 carbon atoms. Examples of alkyl include, collectively or individually, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, n-pentyl, n-hexyl, 2-methylbutyl, 2-methylpentyl, 2-ethylbutyl, 3-methylpentyl, and 4-methylpentyl. Examples of C_1-C_4 alkyl include, collectively or individually, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, and t-butyl.

"Alkenyl" or “alkenylene” is intended to include hydrocarbon chains of either a straight or branched configuration having the specified number of carbon atoms and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain. Examples of alkenyl include, but are not limited to, ethenyl, 1-propenyl, 2-propenyl, 2-butenyl, 3-butenyl, 2-pentenyl, 3, pentenyl, 4-pentenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 2-methyl-2-propenyl, 4-methyl-3-pentenyl, and the like.

"Alkynyl" or “alkynylene” is intended to include hydrocarbon chains of either a straight or branched configuration and one or more carbon-carbon triple bonds which may occur in any stable point along the chain, such as ethynyl, propynyl, butynyl, pentynyl, hexynyl and the like.

"Cycloalkyl" is intended to include saturated ring groups, having the specified number of carbon atoms. For example, "C_3-C_6 cycloalkyl" denotes such as cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

"Alkoxy" or “alkyloxy” represents an alkyl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, n-pentoxy, and s-pentoxy. Similarly, “alkylthio” or “thioalkoxy” represents an alkyl group as defined above with the indicated number of carbon atoms attached through a sulpher bridge.

"Halo" or "halogen" as used herein refers to fluoro, chloro, bromo, and iodo; and "counterion" is used to represent a small, negatively charged species such as chloride, bromide, hydroxide, acetate, sulfate, and the like.

As used herein, "carbocycle" is intended to mean any stable 3- to 7-membered monocyclic or bicyclic or 7- to 13-membered bicyclic or tricyclic, any of which may be

saturated, partially unsaturated, or aromatic. Examples of such carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, cyclooctyl, [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane (decalin), [2.2.2]bicyclooctane, fluorenyl, phenyl, naphthyl, indanyl, adamantyl, or tetrahydronaphthyl (tetralin).

As used herein, the term "heterocycle" or "heterocyclic ring" is intended to mean a stable 5- to 7- membered monocyclic or bicyclic or 7- to 14-membered bicyclic heterocyclic ring which is saturated partially unsaturated or unsaturated (aromatic), and which consists of carbon atoms and 1, 2, 3 or 4 heteroatoms independently selected from the group consisting of N, O and S and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The nitrogen and sulfur heteroatoms may optionally be oxidized. The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom which results in a stable structure. The heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if the resulting compound is stable. If specifically noted, a nitrogen in the heterocycle may optionally be quaternized. It is preferred that when the total number of S and O atoms in the heterocycle exceeds 1, then these heteroatoms are not adjacent to one another. It is preferred that the total number of S and O atoms in the heterocycle is not more than 1.

Examples of heterocycles include, but are not limited to, 1H-indazole, 2-pyrrolidonyl, 2H,6H-1,5,2-dithiazinyl, 2H-pyrrolyl, 3H-indolyl, 4-piperidonyl, 4aH-carbazole, 4H-quinolizinyl, 6H-1,2,5-thiadiazinyl, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazalonyl, carbazolyl, 4a*H*-carbazolyl, b-carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2*H*,6*H*-1,5,2-dithiazinyl, dihydrofuro[2,3-*b*]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, imidazolopyridinyl, 1*H*-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isothiazolopyridinyl, isoxazolyl, isoxazolopyridinyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolopyridinyl, oxazolidinylperimidinyl, oxindolyl, phenanthridinyl, phenanthrolinyl,

phenarsazinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, pteridinyl, piperidonyl, 4-piperidonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolopyridinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, pyrrolyl, quinazolinyl, quinolinyl, 4*H*-quinolizinyl, quinoxalinyl, quinuclidinyl, carbolinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, 6*H*-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thiazolopyridinyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl, and xanthenyl. Preferred heterocycles include, but are not limited to, pyridinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, pyrazinyl, piperazinyl, imidazolyl, indolyl, benzimidazolyl, 1*H*-indazolyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, benzoxazolyl, oxindolyl, benzoxazolinyl, benzthiazolyl, benzisothiazolyl, isatinoyl, isoxazolopyridinyl, isothiazolopyridinyl, thiazolopyridinyl, oxazolopyridinyl, imidazolopyridinyl, and pyrazolopyridinyl. Preferred 5 to 6 membered heterocycles include, but are not limited to, pyridinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, pyrazinyl, piperazinyl, imidazolyl, and oxazolidinyl. Also included are fused ring and spiro compounds containing, for example, the above heterocycles.

As used herein, the term "aryl", or aromatic residue, is intended to mean an aromatic moiety containing the specified number of carbon atoms, such as phenyl and naphthyl.

Also provided herein are pharmaceutical composition comprising compounds provided herein and a pharmaceutically acceptable carrier. Such carriers are media generally accepted in the art for the delivery of biologically active agents to animals, in particular, mammals. Pharmaceutically acceptable carriers are generally formulated according to a number of factors well within the purview of those of ordinary skill in the art to determine and account for. These include, without limitation: the type and nature of the active agent being formulated; the subject to which the agent-containing composition is to be administered; the intended route of administration of the composition; and, the therapeutic indication being targeted.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

Pharmaceutically acceptable carriers include both aqueous and non-aqueous liquid media, as well as a variety of solid and semi-solid dosage forms. Such carriers can include a number of different ingredients and additives in addition to the active agent, such additional ingredients being included in the formulation for a variety of

reasons, e.g., stabilization of the active agent, well known to those of ordinary skill in the art. Descriptions of suitable pharmaceutically acceptable carriers, and factors involved in their selection, are found in a variety of readily available sources, e.g., *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, the contents of which are incorporated herein by reference.

Compounds of this invention are administered, for example, parenterally in various aqueous media such as aqueous dextrose and saline solutions; glycol solutions are also useful carriers. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents, such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or in combination, are suitable stabilizing agents. Also used are citric acid and its salts, and EDTA. In addition, parenteral solutions can contain preservatives such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

Alternatively, the compounds are administered orally in solid dosage forms, such as capsules, tablets and powders; or in liquid forms such as elixirs, syrups, and/or suspensions. Gelatin capsules can be used to contain the active ingredient and a suitable carrier such as but not limited to lactose, starch, magnesium stearate, stearic acid, or cellulose derivatives. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of time. Compressed tablets can be sugar-coated or film-coated to mask any unpleasant taste, or used to protect the active ingredients from the atmosphere, or to allow selective disintegration of the tablet in the gastrointestinal tract.

Further provided herein is a method of delivering compound of this invention to the cells of a mammal in need of antineoplastic treatment, said method comprising contacting the cells with therapeutically effective amounts of the compounds in the presence of the corresponding peptidase. "Therapeutically effective amounts" are any amounts of a compound effective to ameliorate, alleviate, lessen or inhibit the symptoms, progression thereof, or the underlying manifestations of a particular disease, disorder or condition; typically, for *in vivo* treatment, therapeutically effective amounts are from about 0.1 mg of a compound per kg of body weight of the mammal being

treated, to about 1000 mg/kg. Said mammals may be suffering from breast, ovarian, brain, stomach, lung, colon, prostate or liver cancers, or leukemias, lymphomas, carcinomas, sarcomas, or melanomas, as well as other forms of cancers.

The conjugated compounds of the present invention are useful as chemotherapeutic agents in the targeted treatment of cancers. For example, in the treatment of cancers, peptides and antineoplastic agents are conjugated to produce stable conjugates which can be administered to mammals and circulate in the blood stream stable to nonspecific enzymatic degradation, for example neprolysin. Conjugation also reduces the antineoplastic agent's ability to exert its effects on tissue, i.e., healthy, nontarget tissue; such that the agent's toxicity is greatly reduced in comparison to use in its unconjugated, free form. However, once the peptide is cleaved from the antineoplastic agent by one or a combination of membrane-bound and/or cell-secreted peptidases, the agent is released such that it can then exert its desired therapeutic effect on cells in the surrounding area. While multiple peptidases may be involved in removing or processing of the amino acids from the antineoplastic agent, an initiating peptidase cleavage event is required to activate these conjugates. Peptidases, such as the matrixins MMP-2 and MMP-9 and MMP-14, are found in the tumor environment. Hence, conjugation of a matrixin or MMP enzyme-cleavable peptide to an antineoplastic agent offers a novel means of delivering the agent as a therapeutic entity specifically to tumors while reducing the agent's toxicity on healthy, nontarget tissue. However, the conjugate is also designed so that the product of the first proteolytic event is an acceptable substrate for aminopeptidases expressed in the tumor tissue which further remove or process remaining amino acids from the antineoplastic agent. It is known that such aminopeptidases, e.g., dipeptidyl aminopeptidase and neutral aminopeptidase, are expressed in tumor tissue (Pasqualini). Thus, the compounds of the present invention, upon first proteolytic cleavage by a matrix metalloproteinase, are not intended to produce unconjugated Dox.

Peptide/antineoplastic agent conjugates of the present invention are stable in plasma, such stability being demonstrated by a number of means well known in the art, e.g., by incubation in various media (see, e.g., Example 6 hereinbelow). Hence, the conjugates of the present invention can be effectively used as therapeutic entities for administration to mammals. Matrixins and aminopeptidases, are known to be produced

in neoplastic cells, and to be found in the cells, or in their vicinity. Endothelial and stromal cells, which may be found in proximity to the tumor, may also contain peptidase activities that contribute to the delivery of therapeutic entities to the tumor. Such matrixins and aminopeptidases, as described hereinabove, are have been shown to recognize and cleave enzyme-cleavable peptides conjugated to cytotoxic agents herein (see Example 7, hereinbelow), releasing the peptide, in a complete or truncated form, and the agent, with or without amino acids attached. Cleavage releases the cytotoxic antineoplastic agent from the conjugate such that it can then exert its beneficial therapeutic effect on neoplastic cells. Accordingly, conjugation of a matrixin or MMP enzyme-cleavable peptide to a cytotoxic agent affords targeted delivery of the agent as a therapeutic entity specifically to tumors, while minimizing the adverse impact of the agent on healthy, nontarget tissue.

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In another embodiment, the invention describes a method of treating cancer in a patient in need thereof, comprising administrering to said patient a pharmaceutically effective amount of a compound as set forth above, or a pharmaceutically acceptable salt form thereof, wherein the cancer is selected from the group consisting of: carcinoma such as bladder, breast, colon, kidney, liver, lung, including small cell lung cancer, esophagus, gall-bladder, ovary, pancreas, stomach, cervix, thyroid, prostate, and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell-lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias, myelodysplastic syndrome and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma and schwannomas; other tumors, including

melanoma, seminoma, teratocarcinoma, osteosarcoma, xenoderoma pigmentosum, keratoctanthoma, thyroid follicular cancer and Kaposi's sarcoma.

In another embodiment, the invention describes a method of treating cancer in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound of Formula (I) or (Ia) as set forth above, or a pharmaceutically acceptable salt form thereof, in combination (administered together or sequentially) with known anti-cancer treatments such as radiation therapy or with cytostatic or cytotoxic agents, wherein such agents are selected from the group consisting of: DNA interactive agents, such as cisplatin or doxorubicin; topoisomerase II inhibitors, such as etoposide; topoisomerase I inhibitors such as CPT-11 or topotecan; tubulin interacting agents, such as paclitaxel, docetaxel or the epothilones; hormonal agents, such as tamoxifen; thymidilate synthase inhibitors, such as 5-fluorouracil; and anti-metabolites, such as methoxtrexate.

In another embodiment, the invention describes a method treating cancer in a patient in need thereof, comprising administering to said patient a pharmaceutically effective amount of a compound of Formula (I) or (Ia) as set forth above, or a pharmaceutically acceptable salt form thereof, in combination (administered together or sequentially) with known anti-proliferating agents selected from the group consisting of:, altretamine, busulfan, chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan, thiotepa, cladribine, fluorouracil, floxuridine, gemcitabine, thioguanine, pentostatin, methotrexate, 6-mercaptopurine, cytarabine, carmustine, lomustine, streptozotocin, carboplatin, cisplatin, oxaliplatin, iproplatin, tetraplatin, lobaplatin, JM216, JM335, fludarabine, aminoglutethimide, flutamide, goserelin, leuprolide, megestrol acetate, cyproterone acetate, tamoxifen, anastrozole, bicalutamide, dexamethasone, diethylstilbestrol, prednisone, bleomycin, dactinomycin, daunorubicin, doxirubicin, idarubicin, mitoxantrone, losoxantrone, mitomycin-c, plicamycin, paclitaxel, docetaxel, CPT-11, epothilones , topotecan, irinotecan, 9-amino camptothecan, 9-nitro camptothecan, GS-211, etoposide, teniposide, vinblastine, vincristine, vinorelbine, procarbazine, asparaginase, pegaspargase, methoxtrexate, octreotide, estramustine, and hydroxyurea.

As used herein the term "effective amount" means an amount of a compound/composition according to the present invention effective in producing the desired therapeutic effect.

As used herein the term "treating" refers to: (i) preventing a disease, disorder or condition from occurring in an animal which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it; (ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and (iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

As used herein the term "patient" includes both human and other mammals.

As used herein the term "pharmaceutical composition" means a composition comprising a compound of Formula (I) or (Ia) and at least one component selected from the group comprising pharmaceutically acceptable carriers, diluents, adjuvants, excipients, or vehicles, such as preserving agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms. Examples of suspending agents include ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monosterate and gelatin. Examples of suitable carriers, diluents, solvents or vehicles include water, ethanol, polyols, suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Examples of excipients include lactose, milk sugar, sodium citrate, calcium carbonate, dicalcium phosphate phosphate. Examples of disintegrating agents include starch, alginic acids and certain complex silicates. Examples of lubricants include magnesium stearate, sodium lauryl sulphate, talc, as well as high molecular weight polyethylene glycols.

This invention will be better understood when read in light of the following Examples. However, those of ordinary skill in the art will readily understand that the examples are merely illustrative of the invention as defined in the claims which follow thereafter.

EXAMPLES

Conjugation of Peptides to Antineoplastic Compounds

Example 00. Synthesis of Ac-PLGL-Dox.

The peptide acid was synthesized on the solid phase from commercially available Fmoc-Leu-Wang resin (0.40 g, 0.6 mmol). The synthesis was performed on an ABI 433A peptide synthesizer using four equivalents of Fmoc protected amino acids and HBTU activation. The peptide resin was acetylated with acetic anhydride. The peptide was cleaved from the resin with 90% TFA in water for 2h. After solvent removal the peptide was dissolved in H_2O: CH_3CN and freeze dried. Product was confirmed by ES MS 496.3 (M-H). Analytical HPLC on a Metachem Monochrom C18 reverse phase column (50 X 4.6 mm) showed crude peptide to be 85% pure. To this intermediate (0.0199 g, 0.04 mmol) dissolved in DMF (0.2 mL) in a small amber vial was added Pybop (0.0208 g, 0.04 mmol). Doxorubicin hydrochloride (0.0186 g, 0.032 mmol) was added as a suspension in DMF (0.1 mL) followed by diisopropylethylamine (DIEA) (0.0139 mL, 0.08 mmol). The reaction was stirred for 2 h. Solvent was removed under vacuum. Sample was dissolved in H_2O: CH_3CN and purified using a Dynamax C18 reverse phase column (41.4 x 250 mm) with a linear gradient from 30-50% acetonitrile, 0.05% ammonium acetate over 20 minutes with a flow rate of 45 mL/minute. Fractions were pooled and freeze dried to afford the purified peptide-Dox conjugate (ES MS 964.6 (M-H)).

Solid Phase Synthesis of Doxorubicin Conjugates

Example 47: Synthesis of Ac-PLGLYL-Dox.

The peptide acid was synthesized on the solid phase from commercially available Fmoc-Leu-Wang resin (0.42 g, 0.25 mmol). The synthesis was performed on an ABI 433A peptide synthesizer using four equivalents of Fmoc protected amino acids and

HBTU activation. The peptide resin was acetylated with acetic anhydride. The peptide was cleaved from the resin with 90% TFA in water for 2h. After solvent removal the peptide was dissolved in H_2O: CH_3CN and freeze dried. Product was confirmed by ES MS 717.4 (M+H). Analytical HPLC on a Metachem Monochrom C18 reverse phase column (50 X 4.6 mm) showed crude peptide to be 80% pure. To this intermediate (0.0286 g, 0.04 mmol) dissolved in DMF (0.2 mL) in a small amber vial was added PyBop (0.0208 g, 0.04 mmol). Doxorubicin hydrochloride (0.0186 g, 0.032 mmol) was added as a suspension in DMF (0.1 mL) followed by diisopropylethylamine (DIEA) (0.0139 mL, 0.08 mmol). The reaction was stirred for 2 h. Solvent was removed under vacuum. Sample was dissolved in H_2O: CH_3CN and purified using a Dynamax C18 reverse phase column (41.4 x 250 mm) with a linear gradient from 35-55% acetonitrile, 0.05% ammonium acetate over 20 minutes with a flow rate of 45 mL/minute. Fractions were pooled and freeze dried to afford the purified peptide-Dox conjugate
(ES MS 1240.7 (M-H)).

Example 116: Synthesis of Ac-PLG-Hof-Orn-L-Dox.

The peptide acid (Ac-PLG-Hof-Orn(allyl)-L-COOH) was synthesized on the solid phase from commercially available Fmoc-Leu-Wang resin (0.28 g, 0.25 mmol). The synthesis was performed on an ABI 433A peptide synthesizer using four equivalents of Fmoc protected amino acids and HBTU activation. The peptide resin was acetylated with acetic anhydride. The peptide was cleaved from the resin with 90% TFA in water for 2h. After solvent removal the peptide was dissolved in H_2O: CH_3CN and freeze dried. Product was confirmed by ES MS 800.7 $(M+H)^+$, 822.7 $(M+Na)^+$. Analytical HPLC on a Metachem Monochrom C18 reverse phase column (50 X 4.6 mm) showed crude peptide to be 90% pure. To this intermediate (0.320 g, 0.4 mmol) dissolved in DMF (2.0 mL) in a small amber vial was added PyBop (0.204 g, 0.4 mmol). Doxorubicin hydrochloride (0.148 g, 0.26 mmol) was added as a suspension in DMF (1.0 mL) followed by diisopropylethylamine (DIEA) (0.28 mL, 1.6 mmol). The reaction was stirred for 2.5 h. Solvent was removed under vacuum. Sample was dissolved in H_2O: CH_3CN and purified using a Phenomenex LUNA C18 reverse phase column (250 X 21.2 mm) with a linear gradient from 45-55% acetonitrile, 0.05% ammonium acetate over 30 minutes with a flow rate of 18 mL/minute. Fractions were pooled and freeze dried to afford the purified Ac-

PLG-Hof-Orn(allyl)-L-Dox (ES MS 1325.4 $(M+H)^+$, 911.4 $(M+H-414)^+$). Side chain protected peptide (0.076 g, 0.06 mmol) was dissolved in dry DCM (7 mL) under Ar_2. $[(Ph_3)P]_4Pd$ (0.014 g, 0.012 mmol) in DCM (1mL) was added followed by morpholine (0.052 mL, 0.6 mmol). The reaction was stirred at rt for 2h and monitored by HPLC. Product was precipitated from EtOAc and washed with EtOAc (2x). Solvent was removed with a N_2 flow. Unprotected conjugate (Ac-PLG-Hof-OrnL-Dox) was purified using a Phenomenex LUNA C18 reverse phase column (250 X 21.2 mm) with a linear gradient from 25-40% acetonitrile, 0.05% ammonium acetate over 30 minutes with a flow rate of 18 mL/minute. Fractions were pooled and freeze dried to afford the purified product (95% pure) (ES MS 1241.9 $(M+H)^+$, 827.7 $(M+H-414)^+$).

Alternate Solid Phase Synthesis of Doxorubicin Conjugates

Example 11: Synthesis of Acp-PLGLL-Dox.

Acp = 4-(2-aminoethyl)-1-carboxymethyl piperazine. The Fmoc protected peptide acid (Fmoc-Acp-PLGLL-COOH) was synthesized on the solid phase from commercially available Fmoc-Leu-Wang resin (1.6 g, 1.0 mmol). The synthesis of PLGLL-resin was performed on an ABI 433A peptide synthesizer using three equivalents of Fmoc protected amino acids and HBTU activation. A portion of the peptide resin (0.18 g, 0.1 mmol) was then coupled to Fmoc-Acp dihydrochloride (0.193 g, 0.4 mmol) with HBTU (0.152 g, 0.4 mmol) and DIEA (0.143 mL, 0.8 mmol) in DMF (2 mL) for 2 h. The peptide was cleaved from the resin with 90% TFA in water for 2h. After solvent removal the peptide was dissolved in H_2O: CH_3CN and freeze dried. To this intermediate (0.036 g, 0.04 mmol) dissolved in DMF (0.2 mL) in a small amber vial was added PyBop (0.021 g, 0.04 mmol). Doxorubicin hydrochloride (0.018 g, 0.032 mmol) was added as a suspension in DMF (0.1 mL) followed by diisopropylethylamine (DIEA) (0.014 mL, 0.08 mmol). The reaction was stirred for 2 h. Solvent was removed under vacuum. Sample was dissolved in H_2O: CH_3CN and purified using a Phenomenex LUNA C18 reverse phase column (250 X 21.2 mm) with a linear gradient from 20-50% acetonitrile, 0.05% ammonium acetate over 30 minutes with a flow rate of 18 mL/minute. Fractions were pooled and freeze dried to afford the Fmoc-Acp-PLGLL-Dox (ES MS 1428.9 $(M+H)^+$, 1014.7 $(M+H-414)^+$). Fmoc protected peptide (0.020 g, 0.014 mmol) was dissolved in a cold solution of 50%

diethylamine in DCM (6 mL). The reaction was stirred protected from light at 0° for 3h. The solvent was removed under vacuum. DCM was added to redissolve the sample and was removed under vacuum 4X. The sample was dried further with a flow of N_2. The sample was then washed with Hex:Et_2O, 1:1 5X followed by evaporation under vacuum and a final flow of N_2. Sample was dissolved in acetate buffer: CH_3CN and purified using a Phenomenex LUNA C18 reverse phase column (250 X 21.2 mm) with a linear gradient from 15-50% acetonitrile, 0.05% ammonium acetate over 35 minutes with a flow rate of 18 mL/minute. Fractions were pooled and freeze dried to afford the purified (90% pure) Acp-PLGLL-Dox (ES MS 1207 $(M+H)^+$, 793 $(M+H-414)^+$).

For examples of this invention where unusual amino acids are coupled to the chemotherapeutic agent, for example doxorubicin, the requisite solid support is frequently not commercially available. The following example illustrates how the modified support is prepared in these cases.

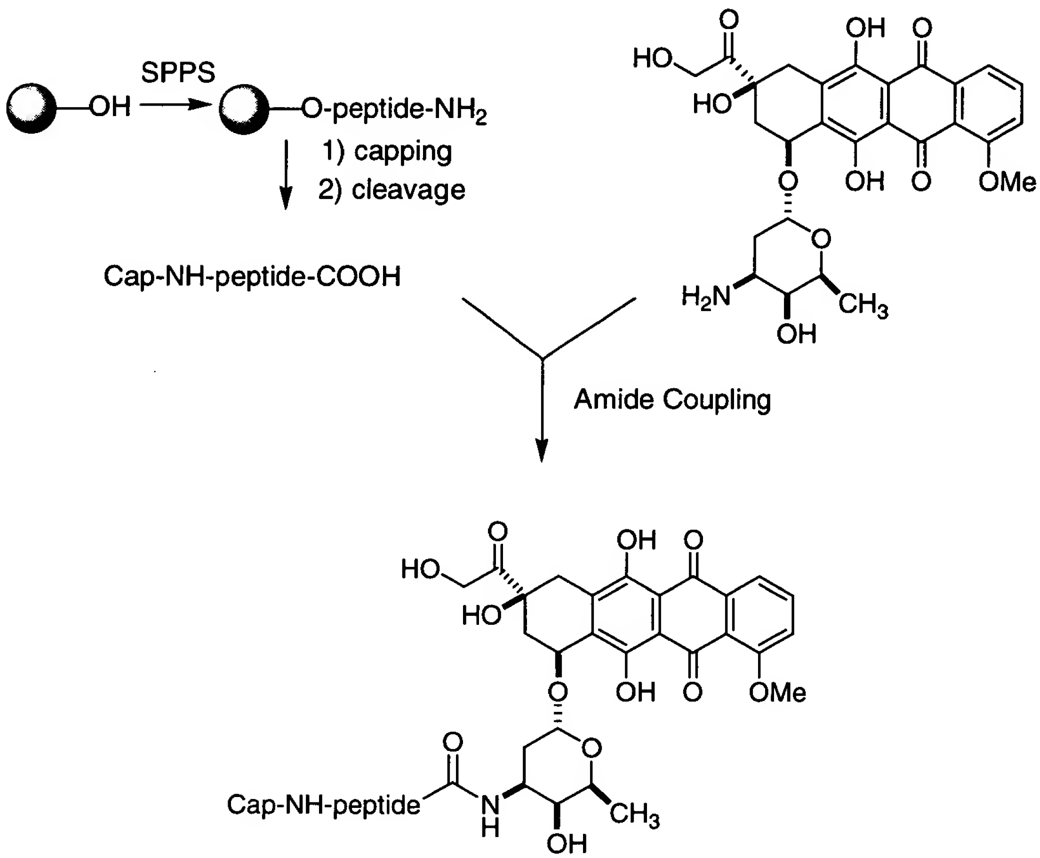
Example 182: Synthesis of Ac-PLG-Hof-Y-Hol-Dox.

Coupling of unnatural amino acids to solid support.

Triphenyl phosphine (4.78 g, 18.25 mmol) was dissolved in DMF (100 mL) and the solution was cooled to 0°C. Wang resin (5.2 g, 4.45 mmol) was added, the reaction was stirred for 10 minutes followed by addition of carbon tetrabromide (6.06 g, 18.25 mmol). The reaction was stirred for 5 h. The resin was washed and dried. A portion of the resin (0.281 g, 0.25 mmol) was swelled in DMF (2.5 mL), Fmoc-Hol (0.138g, 0.375 mmol) was added, followed by DIEA (0.065 mL, 0.375 mmol) and Cesium iodide (0.065 g, 0.25 mmol). The reaction was rocked overnight. The resin was washed and completion of reaction was corroborated by ninhydrin test. The resin was then transferred to the peptide synthesizer for subsequent couplings. Coupling to Doxorubicin was done as in Example 47. Ac-PLG-Hof-Y-Hol-Dox (ES MS 1326.3 $(M+Na)^+$, 890.4 $(M+H-414)^+$).

Scheme 1

Solid Phase Synthesis of Doxorubicin Conjugates



Solution Phase Synthesis of Conjugates

Example 104: Synthesis of Ac-Pro-Leu-Gly-Hof-Gly(morpholinylpropyl)-Leu-Dox (Scheme 2)

(Step 1a): To a mixture of Z-Leu-OH (2.65 g, 10 mmol), H-Gly-OtBu hydrochloride (1.7 g, 10 mmol) and EDCI (2.3 g, 12 mmol) in 200 mL CH_2Cl_2 was added diisopropylethylamine (3.0 mL) slowly at 0 °C. The resulted mixture was stirred at this temperature for 30 min and at room temperature for 2 hrs. Then, the reaction mixture was diluted with CH_2Cl_2, washed with 1N HCl solution, Sat. $NaHCO_3$, water and brine, and dried over $MgSO_4$. After filtration and concentration, the desired dipeptide Z-Leu-Gly-OtBu was obtained as white solid (3.75g, >95%). MS found $(M+1)^+$ 379.2.

(Step 1b): The dipeptide obtained from (Step 1a) (3.75 g, 10 mmol) was dissolved in methanol (200 mL), and the mixture was hydrogenated in the presence of catalytic amount of Pd/C (0.1 mol%) and a few drops of 4N HCl in dioxane at 1 atm for 3 hrs. The reaction mixture was filtered, concentrated and dried.

The amine obtained above was dissolved in CH_2Cl_2 (500 mL), and to this mixture were added Ac-Pro-OH (1.57 g, 10 mmol), EDCI (2.3 g, 12 mmol), catalytic amount of HOBT (100 mg), and diisopropylethylamine (4.0 mL). The mixture was stirred at room temperature for 3.5 hrs. Then, the reaction mixture was diluted with CH_2Cl_2, washed with 1N HCl solution, Sat. $NaHCO_3$, water and brine, and dried over $MgSO_4$. Chromatography on silica gel (20% EtOAc in hexane) yielded the desired tripeptide Ac-Pro-Leu-Gly-OtBu as white solid(3.63g, 95%) . MS found $(M+1)^+$ 384.3.

(Step 1c): The tripeptide obtained from (Step 1b) (3.63g, 9.5 mmol) was dissolved in CH_2Cl_2 (100 mL), and TFA (100 mL) was added slowly at 0 °C. The mixture was stirred at 0 °C for 15 min. and room temperature for 2 hrs. Evaporation of solvent provided the desired acid Ac-Pro-Leu-Gly-OH as white solid (3.08g, >95%). MS found $(M+1)^+$ 328.2.

(Step 2a): A mixture of Z-Glu-OtBu (3.0 g, 8.9 mmol), morpholine (2.0 mL,23 mmol), EDCI (2.22 g, 11.6 mmol),), catalytic amount of HOBT (50 mg), and diisopropylethylamine (2.0 mL) in THF (60 mL) was stirred at room temperature for 3 hrs. Most of the solvent was removed, the residue was dissolved in EtOAc (100 mL) and washed with 1N HCl solution, Sat. $NaHCO_3$, water, brine, and dried over $MgSO_4$. Evaporation of solvent provided the desired compound as white solid (3.6g, >95%). MS found $(M+1)^+$ 407.2.

(Step 2b): The material from (Step 2a) (3.5 g, 8.62 mmol) was dissolved in THF (50 mL). To this mixture was added BH_3 THF (1.0 M, 10mL) and the resulted mixture was stirred at reflux for 1.5 hr and room temperature for 30 min. Solvent was removed, the residue was dissolved in EtOAc (100 mL) and washed with Sat. $NaHCO_3$, water, brine.

Chromatography on silica gel (60% EtOAc in hexane) yielded the desired Z-Gly(morpholinylpropyl)-OtBu as white solid (2.7g, 81%). MS found $(M+1)^+$ 393.1.

(Step 2c): Following a procedure analogous to (Step 1c) (2.7g, 6.89 mmol), the material from (Step 2b) was treated with TFA to give acid Z-Gly(morpholinylpropyl)-OH as white solid (2.3g, >95%). MS found $(M-1)^-$ 335.1.

(Step 2d): The material obtained from (Step 2c) (392 mg, 1.0 mmol) was dissolved in DMF (10 mL). To this mixture were added H-Leu-OMe hydrochloride salt (182 mg, 1.0 mmol), BOP (442 mg, 1.0 mmol) and DIEA (0.52 mL, 3.0 mmol). The resulted mixture was stirred at room temperature for 2 hrs. Most of the solvent was removed, and the residue was diluted with EtOAc (80 mL), washed with 1N HCl solution, Sat. $NaHCO_3$, water, brine, and dried over MgSO4. After HPLC purification ($CNCH_3/H_2O$), the desired dipeptide Z-Gly(morpholinylpropyl)-Leu-OMe was obtained as white solid (393mg, 85%). MS found $(M+1)^+$ 464.6.

(Step 2e): The dipeptide obtained from (Step 2d) (393mg, 0.85 mmol) was dissolved in methanol (100 mL), and the mixture was hydrogenated in the present of catalytic amount of Pd/C (0.1 mol%) and a few drops of 4N HCl in dioxane at 1 atm for 3 hrs. The reaction mixture was filtered, concentrated and dried.

Following a procedure analogous to (Step 2d), the material from above was coupled with Boc-Hof-OH to give desired tripeptide Boc-Hof-Gly(morpholinylpropyl)-Leu-OMe as white solid (381mg, 76%). MS found $(M+1)^+$ 591.4.

(Step 2f): Following a procedure analogous to (Step 1c), the material obtained from (Step 2e) (381mg, 0.65 mmol) was treated with TFA to provide the corresponding amine. MS found $(M+1)^+$ 491.4.

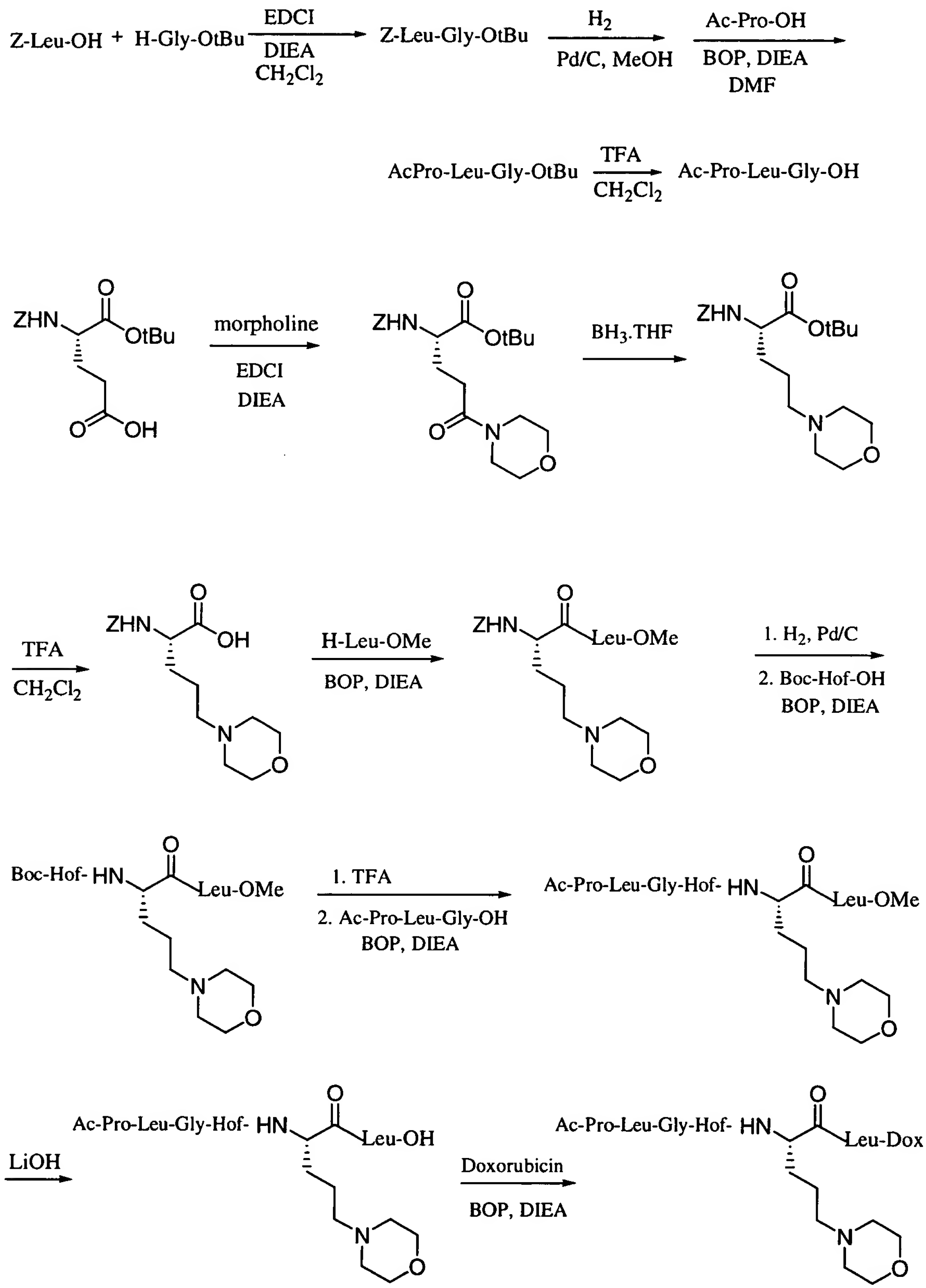
Following a procedure analogous to (Step 2d), the material from above was coupled with tripeptide Ac-Pro-Leu-Gly-OH to give the desired hexapeptide Ac-Pro-

Leu-Gly-Hof-Gly(morpholinylpropyl)-Leu-OMe as white solid (437mg, 84%). MS found $(M+1)^+$ 800.5.

(Step 2g): To a solution of the material (400 mg, 0.5 mmol) obtained from (Step 2f) in THF (5 mL) at 0 °C was added 1N LiOH solution (5 mL). After stirring at this temperature for 3 hrs, the reaction mixture was acidified with 1N HCl (5 mL) to pH 5. Solvent was removed and the mixture was purified by HPLC ($CNCH_3/H_2O$). The desired hexapeptide was obtained as white solid (337mg, 86%). MS found $(M-1)^-$ 784.5.

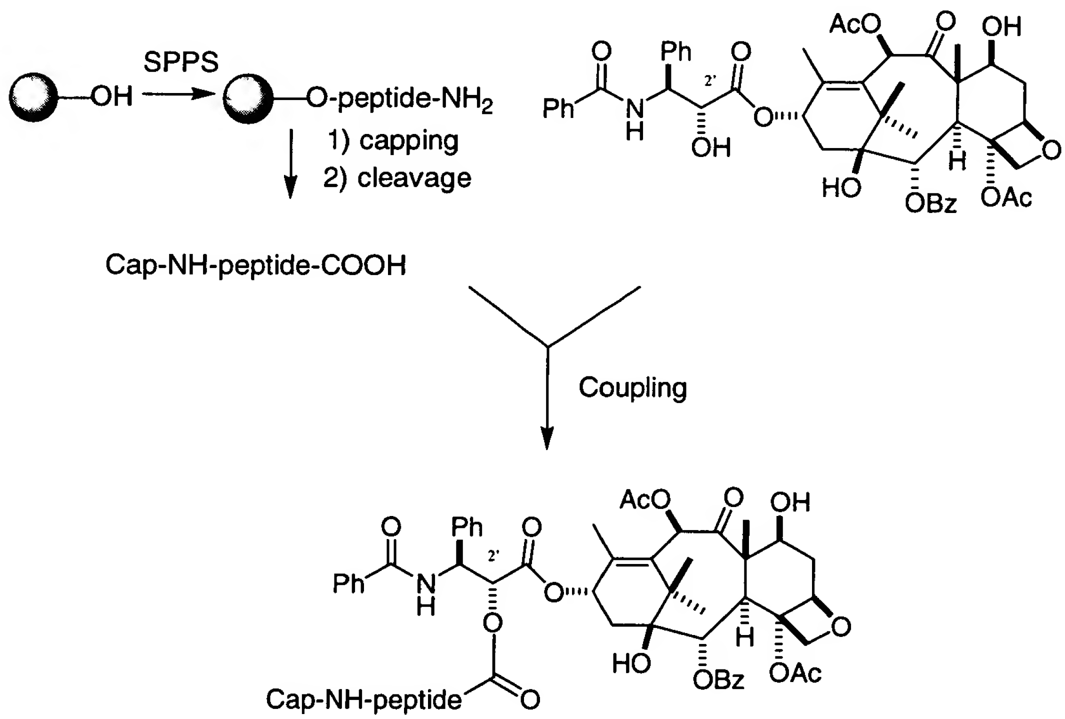
(Step 2h): To a solution of the material obtained from (Step 2g) (39 mg, 0.05 mmol) in DMF (5 mL) at 0 °C were added BOP (27 mg, 0.06 mmol) and DIEA (0.05 mL). After stirring at this temperature for 5 min., doxorubicin hydrochloride (30 mg, 0.05 mmol) was added to the above mixture. The resulted mixture was stirred in dark at 0 °C for 1 hr and at room temperature for 2 hrs. Most of the solvent was removed and the residue was purified by HPLC [CH_3CN (0.1% NH_4Ac)/H_2O(0.1% NH_4Ac)]). MS found $(M-1)^-$ 1309.1. (Note: There are two HPLC peaks with the desired mass. These may be the two diastereomers caused by racemization during the coupling).

Scheme 2: Solution Phase Synthesis of a Representative Doxorubicin Conjugate



Synthetic methodology is known in the literature for the selective acylation of the important chemotherapeutic agent paclitaxel. For example , L-alanine has been introduced onto the 2' hydroxyl of paclitaxel (Sundfor, 1998). Should ester prove to have suboptimal stability properties, it is known in the art that a carbamate-based linker strategy will generate more stable conjugates (de Groot). This methodology has previously been used to deliver paclitaxel to tumors using plasmin; however, appropriate engineering of the peptide sequence as disclosed in this invention should generate conjugates that are cleavable by MMPs.

Scheme 3: Synthesis of Paclitaxel Conjugates



It has been shown in the literature that peptides may be attached to *Vinca* alkaloids, such as vinblastine and vincristine. For example, the carbomethoxy group of vinblastine may be selectively activated and attached to the N-terminus of a peptide chain (Kandukuri). A skilled artisan could combine this technology with the peptide sequences of this invention to generate MMP cleavable vinca alokaloid conjugates.

Example 1000: Evaluation of Stability of Conjugates in Blood

The stability of doxorubicin conjugated peptides in human or nude mouse blood was evaluated by reverse phase HPLC with fluorescence detection after an 80%

acetonitrile extraction. Individual peptides are prepared as 60 µmolar solutions in Hepes buffer pH 7.5 (50 mM), with $CaCl_2$ (10 mM), Brij-35 (0.1%), followed by dilution to 10 µmolar in fresh heparinized whole blood or buffer. Solutions are incubated (37° C) with slow continuous rocking. 50 µl reactions are terminated at designated times ranging from 1 minute to 24 hours by vortexing into 200 µl acetonitrile. After a brief centrifugation (1 min, 14,000 x g) to pellet the precipitate, the acetonitrile is collected and evaporated to dry under a flow of nitrogen. Extracted samples are resuspended in 50 µl acetonitrile, followed by 100 µl distilled H_2O, and transferred to HPLC autoinjector vials. Samples are chromatographed using a Nova-Pak C18 column (3.9 x 150 mm; WAT086344, Waters Corp. Milford, Ma), with a 12 minute linear gradient from 33.3 to 77.7 % acetonitrile, 0.1% TFA, using a flow rate of 1 ml/min. A scanning fluorescence detector (# 474, Waters Corp) monitoring 480 nm excitation, 580 nm emission quantitates AUC of peaks of interest; mass is extrapolated from a standard curve generated under matching conditions. Results are presented in Table 1, below.

Table 1
Conjugate Stability Summary in Blood After 5.5 Hours
(Percent of Control (In Buffer, T=0))

	Buffer	Human Blood	Nude Mouse Blood
Ac-PLG-LYAL-Dox	91.3 %	37.5 %	20.0 %
Ac-PLG-LLAL-Dox	102.0	55.6	19.2
Ac-PLG-LAL-Dox	96.8	49.1	9.0
Ac-PLG-LYL-Dox	112	90.1	---
Ac-PLG-LL-Dox	106	87.2	63.8
Ac-GPLG-LL-Dox	105	42.6	25.8
Ac-GPLG-LAL-Dox	92.2	15.4	5.8
Ac-PLG-L-Dox	99.2	74.7	68.2
Ac-GPLG-L-Dox	106	10.2	5.9

Evaluation of Conjugates as MMP and Neprilysin Substrates

Compounds of this invention should be good substrates for specific MMPs but should not be substrates for related proteases which are not exclusively expressed in the

tumor environment. An example of such an unwanted protease activity is neprilysin, which was identified as a major metalloprotease in several human tumor cell lines. Neprilysin is expressed in kidney, macrophages, and brain tissues (Li et al.). To enhance the targetting of conjugates to tumor tissue, conjugates were tested as substrates for MMPs and neprilysin. Compounds of this invention have k_{cat}/K_m > 1000 mM^{-1} s^{-1} when assayed using a relevant MMP and have k_{cat}/K_m < 1000 mM^{-1} s^{-1} when assayed using neprilysin.

Example 1001

Cleavage of doxorubicin-peptide conjugates with MMPs and Neprilysin

Doxorubicin-peptide conjugates were dissolved in DMSO to 10 mM. The conjugate was initially diluted to 10 μM in Metalloprotease Reaction Buffer (50 mM Hepes pH 7.5, 0.1% Brij 35, 10 mM CaCl2). MMP2, 9, or 14, or neprilysin were diluted to a final concentration of 10 μM into Metalloprotease Reaction Buffer plus 400mM NaCl. In a reaction volume of 1 ml, the dox-conjugate was diluted to 1 μM in Metalloprotease reaction buffer. The reaction was equilibrated at 37^{o} C. Enzyme was added to initiate reaction, 2 nM MMP-9, or 4 nM MMP-2,or 2.5 nM MMP-14 or 10 nM neprilysin. 100 μL aliquots were withdrawn at indicated time points (0, 5, 10, 15, 20, 30, 40, 50, 60 minutes) and quenched with 10 μL of 0.5 M EDTA. The conjugates and products were separated by reverse phase HPLC on a Waters Alliance HPLC system (2690 separations module with 474 scanning fluorescence detector). A 20 μL sample was loaded on a 3.9 mm X 150 mm Waters C18 Novapak column, and eluted with a 12 minute gradient from 27% to 63% acetonitrile / 0.1 % TFA at 1 ml/minute. Doxorubicin containing peaks were detected by fluorescence, excitation at 480 nM, emission at 580 nM. Peak areas were integrated and the substrate peak area was plotted against time. Data was fitted to a single exponential decay curve where $y = A_o e[-kt]$. A_O is the initial value of y, the area of the substrate peak, and k is the rate constant of the reaction. Since the reaction was run under first order conditions (substrate<<Km), k_{cat}/K_m can be calculated from $k_{cat}/K_m = k/[E_t]$. Results are presented in Table 2.

Table 2

	Enzyme*			
	MMP-9	**MMP-2**	**MMP-14**	**Neprilysin**
AcPLG-LYL-Dox	390,000	88,000	---	22,000
AcPLG-LYAL-Dox	296,000	190,000	134,000	388,000
AcPLG-LAAL-Dox	165,000	110,000	---	120,000
AcPLG-LLSL-Dox	149,000	103,000	---	82,000
AcPLG-LLAL-Dox	130,000	63,000	---	100,000
AcPLG-LL-Dox	130,000	18,000	4,100	22,000
AcGPLG-LL-Dox	95,000	30,000	---	20,000
AcGPLG-LY-Dox	110,000	40,000	---	19,000
AcPLG-LAL-Dox	24,000	53,000	---	49,000
AcGPLG-LAL-Dox	19,000	86,000	---	42,000
AcPLG-HofYL-Dox	34,000	>120,000	>120,000	<1000
SucPLG-HofYL-Dox	>120,000	>120,000	>120,000	<1000
AcPLG-HofOrnL-Dox	26,000	136,000	>120,000	<1000

* Where more than one measurement was taken, the value given is an average of the multiple measurements.

Example 1002

Evaluation of conjugates as aminopeptidase substrates.

Conjugates were incubated with 1 nM MMP2 for 3 hours at 37^{o} C in 50 mM HEPES, 10 mM $CaCl_2$, 0.1% Brij, pH 7.5 to generate LYL-Dox, the post-MMP product. Aminopeptidase N (Boehringer Mannheim #102 768) was then added to 12.5 mUnits/ml to initiate post-MMP processing. Aliquots of the reaction mixture (0.045 mL) were removed after various times (3, 6, 9, 15, 20, 30, and 100 min) and added to tubes with 0.005 ml 0.5 mM EDTA to inhibit aminopeptidase activity. One half of the aliquot from each time was separated on a Novapak C18 column (3.9 x 150 mm) at a flow rate of 1 ml/min using the gradient outlined in Table 3. For the HPLC gradients: Solvent A is 14 mM NaPi, 0.5 mM triethylamine, pH 4.2; Solvent B is 50% A, 50% Acetonitrile; and Solvent C is Acetonitrile. The fractional composition was determined using the integrated peak areas.

Table 3. HPLC Gradient

Time, min	A, %	B, %	C, %
0	50	50	0
12	0	100	0
18	0	100	0
19	0	0	100
22	0	0	100
22.5	50	50	0
27	50	50	0

Example 1003

Evaluation of Cytotoxicity of Conjugates/

The conjugates were tested for cytotoxic effect against the HT1080 cell line, which expresses multiple MMPs. Cells can vary significantly in expression of active MMPs; thus, a given cell line may not be optimal for the evaluation of a given conjugate. HT1080 cells in culture have significant levels of MMPs 2, 9, and 14 and are consequently especially suitable for the evaluation of conjugates that are substrates for that enzyme.

The cell line was grown in tissue MEM with Earl's salts containing 10% fetal bovine serum (FBS). On day one, 500 cells were seeded into 96 well plates in 200ul of cell culture medium that containing 10% FBS which had been stripped of bovine gelatinases by prior passage over a gelatin-sepharose column. On day two, peptidyl-Doxorubicin conjugates and Doxorubicin as a control were added to the plates. The cells were incubated for three days at 37^{o} C, 5% CO2 in a tissue culture cell incubator. MTS reagent was added to each microplate well using the manufacturer's instructions (ref). The plates were incubated for 2 hours at 37^{o} C, 5% CO2. The plates were read on a Molecular Devices Spectropmax 250 plate reader at 490nM. The viability of the cells in each well was then calculated for each concentration of compound tested and compared to the control wells where no compound was added. Representative compounds of the present invention have demonstrated EC50 for cell kill </= 10 µM in this assay; more preferably representaitve compounds of the present invention have demonstrated EC50 for cell kill < 1 µM .

Table 4

Cytotoxicity of Conjugates on HT1080 Cells

Compound	EC_{50} (nM)
Doxorubicin	8-9
Ac-PLG~LYAL-Dox	<10,000
Ac-PLG~LLAL-Dox	<10,000
Ac-PLG-LL-Dox	<10,000
Ac-PLG~LAL-Dox	<10,000

Alternatively, delivery of active cytotoxic agent may be assessed by incubating the conjugates with cells and assaying the levels of active species by HPLC. An example of this method of evaluation follows.

Example 1004: Analysis of processing by HT1080 cultures

Actively growing HT1080 cells are seeded in a 12 well plate at 2×10^5 cells per well in DMEM with 10% serum. On the next day, media is removed and cells are washed twice with PBS. 1.5 of DMEM containing 0.1% BSA, 1 μM Ac-PLG-HofK(Me2)L-Dox, and 40 nM PMA is then added to each well. A broad spectrum MMP inhibitor is added to some samples so that the amount of processing that is due to MMPs can be determined. At the indicated times, 0.1 ml aliquots are removed, added to 0.4 ml acetonitrile, vortexed, and centrifuged for 2 minutes. 0.4 of cleared supernatant is removed and dried using a nitrogen stream. The dried pellet is suspended in 0.12 ml of HPLC Buffer A and analyzed as in Example 1000.

Results from a typical analysis are summarized in Table 5. At the times used in this experiment, the only detectable metabolite is L-Dox. HofK(Me2)L-Dox and K(Me2)L-Dox are not detected since they are rapidly converted to L-Dox. At later times, Dox is formed from L-Dox. Processing is greatly reduced by the MMP inhibitor showing that MMPs are the major processing enzymes in these cells.

Table 5.
Analysis of processing in HT1080 cultures

	Fraction of L-Dox	
Time, hours	Minus MMP inhibitor	Plus MMP inhibitor
0	0	0
3	0.10	0.004
5.5	0.20	0.01
8	0.46	0.02

Example 1005: Chromatographic studies designed to evaluate preferential accumulation of Dox in HT1080 xenografts relative to heart and plasma tissues are described as follows.

Conjugate administration and tissue harvesting:

HT1080 tumors are transplanted into naïve Swiss Nude mice from tumor xenograft fragments and allowed to grow in vivo for 1 week.. Experimental Dox-conjugates are dissolved in N,N-Dimethyl-acetamide (DMAC) and then diluted with water to yield the desired conjugate concentration in 10 % DMAC. 0.2 ml conjugate solution is then injected into tail veins. At various times following injection, three mice are anesthetized with CO_2 and blood is collected by cardiac puncture in a syringe containing 0.1 ml Na Citrate. Blood is transferred to a microfuge tube and centrifuged for 2 min in an Eppendorf centrifuge. 0.3 ml of plasma is then transferred to a fresh tube and frozen using liquid nitrogen. Following death, the tumor, left kidney, and heart are removed and frozen using liquid nitrogen. Tissues are stored at –80 C until extraction.

Tissue extraction:

Samples are thawed, weighed and minced with scissors and cold, citrated mouse plasma (Cocalico Biological (#30-0931) is added. Iced slurries are homogenized for about 1 min. with IKA Ultra-Turrex homogenizer and 0.5 ml is then transferred to a microfuge tube. 0.1 ml of 33% Silver nitrate solution is added immediately after homogenization. 0.5 ml of acetonitrile is then added and the resulting mixture is vortexed briefly, mixed for 15 min, and centrifuged for 5 min. The supernatant is

transferred to a fresh tube, dried with a nitrogen stream at 37 degrees C, and stored a –80 degrees C.

Separation, identification and quantification of Dox and Dox-containing compounds in extracted samples:

0.06 ml acetonitrile is added to the thawed, dried samples and vortexed briefly. 0.6 ml Buffer A is then added, and vortexed briefly followed by a 1 min. sonication in a water bath. Samples are centrifuged for 10 min to remove insoluble material and the cleared supernatant is diluted with 60 UL Buffer A to match the composition ;of the HPLC buffer upon injection. 0.1 ml is then injected onto a Novapak C18 column (3.9 x 150 mm) at a flow rate of 1 ml/min and eluted with the following gradient:

Time	% A	%B	%C
0	50	50	0
12	0	100	0
18	0	100	0
19	0	0	100
33	0	0	100
34	50	50	0
40	50	50	0 (end of run)

Buffer A: 14 mM NaPi, 0.5 mM Triethylamine, pH 4.2

Buffer B: 50% Buffer A, 50% Acetonitrile

Buffer C: 100% Acetonitrile

Detection method is fluorescence, with excitation of 480mm, emission of 580mm.

Samples from mouse tissues typically show three major peaks that co-migrate with parental conjugate, authentic Leu-dox and Doxorubicin. To calculate the amount of these species, peak areas from tissue samples are converted to pmol/injection using the equation derived from a Dox standard curve. Pmol/injection values are then multiplied by 2.4 to yield pmol/sample. Pmol/sample values are divided by the tissue mass analyzed (plasma = 0.3 ml, tumor=0.086 mg, heart, kidney, liver = 0.042 mg) to yield pmol/mass. Average and standard errors are then calculated from pmol/mass values for

the 3 samples from each time and tissue. Concentration –time curves, PK parameters, and relative tissue distribution are determined from these average pmol/mass values.

Additional examples of this invention have been prepared using the methods desclosed herein and evaluated using the methodology described in the Examples above. Representatives of this invention are given in Table 6a through 6g..

Table 6a

Example	Cap—P1—P1'—P2'—X--Doxorubicin	M/Z:
Example 164	4-(2-(5,6,7,8-tetrahydronaphthenyl))butyl -G-Hof-Y-L-Dox	1256.6 (M+H+H2O)

Table 6b

Example	Cap—P2—P1—P1'—P2'—X--Doxorubicin	M/Z:
Example 1	4-methoxy-benzenesulfonyl- β -Ala-G-Hof-Y-L-Dox	1277.1 (M-H)
Example 2	1,2-C_6H_4 $(CO)_2$- H-G-Hof-Y-L-Dox	1305.5 (M+H)
Example 41	acetyl - L-G-L-Y-L-Dox	1145.8 (M+H)
Example 42	cyclopropylcarbonyl - L-G-L-Y-L-Dox	1171.7 (M+H)
Example 43	cyclobutylcarbonyl - L-G-L-Y-L-Dox	1185.7 (M+H)
Example 44	pivaloyl - L-G-L-Y-L-Dox	1187.8 (M+H)

Table 6c

Example	Cap—P3—P2—P1—P1'—X--Doxorubicin	M/Z:
Example 3	Acetyl - P-L-G-L-L-Dox	1079
Example 4	Acetyl - P-(R)L-G-L-L-Dox	1079
Example 5	Acetyl - P -(β -Ala) -G-L-L-Dox	1037
Example 6	Acetyl - P -(γ-Abu) -G-L-L-Dox	1051
Example 7	Acetyl -P-Cha-G-L-L-Dox	1119 (M+Na)
Example 8	P-L-G-L-L-Dox	1059.5 (M+Na)
Example 9	$MeOCH_2CH_2OCH_2C(=O)$- P-L-G-L-L-Dox	1153
Example 10	$MeOCH_2CH_2OCH_2CH_2OCH_2C(=O)$- P-L-G-L-L-Dox	1197.9 (M+H)
Example 11	$H_2NCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)$- P-L-G-L-L-Dox	1206
Example 12	$AcHNCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)$- P-L-G-L-L-Dox	1248
Example 13	$AcN(CH_2CH_2)_2NCH_2C(=O)$- P-L-G-L-L-Dox	1205
Example 17	Dmg- P-R-Sar-Hof-L-Dox	1227
Example 18	Acetyl-P-H-G-Hof-L-Dox	1151.2 (M+H)
Example 19	Acetyl-P-Orn-G-Hof-L-Dox	1128.4 (M+H)
Example 20	Acetyl-P-Dap-G-Hof-L-Dox	1100
Example 21	Acetyl-P-Cit-G-Hof-L-Dox	1171

Example 22	Acetyl-P-L-G-(O-(3-pyridyl-))Y-L-Dox	1206.523 (M+H)
Example 23	Acetyl-P-L-G-(O-(4-pyridyl-))Y-L-Dox	1206.524 (M+H)
Example 24	Acetyl-P-L-G-(4-aza-)Hof-L-Dox	1128.517 (M+H)
Example 25	Acetyl-P-L-G-(O-benzyl-)S-L-Dox	1141.5 (M-H)
Example 26	Cbz-P-L-G-(O-(4-pyridylmethyl-))Y-L-Dox	1312.8 (M+H)
Example 27	Acetyl -P-L-Sar-L-L-Dox	1093.534 (M+H)
Example 28	Acetyl -P- (N-Me-)L-G-L-L-Dox	1115.518 (M+Na)
Example 29	Acetyl -P- L-G-(N-Me-)L-L-Dox	1115.517 (M+Na)
Example 30	Acetyl -Hyp- L-G-L-L-Dox	1117.494 (M+Na)
Example 31	Acetyl -Tzc-L-G-L-L-Dox	1119.454 (M+Na)
Example 32	Acetyl -(Homo-P)-L-G-L-L-Dox	1115.516 (M+Na)
Example 33	Acetyl -(Homo-P)-L-G- Hof -L-Dox	1163.516 (M+Na)
Example 34	Acetyl -(Homo-P)-Orn-G- Hof -L-Dox	1142.529 (M+Na)
Example 35	Acetyl - Nipecotate -L-G-L-L-Dox	1142.529 (M+Na)
Example 36	Acetyl - Aze-L-G-L-L-Dox	1087.485 (M+Na)
Example 37	Acetyl - Chg - L-G-L-L-Dox	1143.548 (M+Na)
Example 38	Acetyl - P-valerolactam -G-L-L-Dox	1085.468 (M+Na)
Example 39	Acetyl -G-P-L-G-L-F-Dox	1170.9 (M+H)
Example 40	Acetyl -G-P-L-G-F-F-Dox	1204.9 (M+H)
Example 141	Acetyl -(4-fluoro-F)- L-G-L-L-Dox	1226.528 (M+Na)

Table 6d

Example	Cap—P3—P2—P1—P1'—P2'—X--Doxorubicin	M/Z:
Example 46	acetyl - P-L-G-L-A-L-Dox	1148.8 (M-H)
Example 47	acetyl - P-L-G-L-Y-L-Dox	1240.9 (M-H)
Example 48	Peg - P-L-G-L-Y-L-Dox	1360.9 (M+H)
Example 49	$H_3CC(=O)NH$-Peg - P-L-G-L-Y-L-Dox	1388
Example 50	$AcHNCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)$- P-L-G-L-Y-L-Dox	1411.8 (M+H)
Example 51	acetyl - P-L-G-L-S-L-Dox	1166
Example 55	acetyl - P-L-G-L-L-L-Dox	1193.4 (M+H)
Example 101	acetyl - P-L-G-Hof-H-L-Dox	1264.3 (M+H)
Example 102	acetyl - P-L-G-Hof-A-L-Dox	1196.8 (M-H)
Example 103	acetyl - P-L-G-Hof-Y-L-Dox	1288.8 (M-H)
Example 104	acetyl - P-L-G-Hof- (morpholinylpropyl-G) -L-Dox	1311.6 (M+H)
Example 106	succinyl - P-L-G-Hof-Y-L-Dox	1349.6 (M+H)
Example 107	acetyl - P-L-G-Hof- (O-(4-pyridylmethyl)-Y)-L-Dox	1381.8 (M+H)
Example 108	acetyl - P-L-G-(homo-Y)-Y-L-Dox	1304.6 (M-H)
Example 109	acetyl - P-L-G-(4-aza-Hof)-Y-L-Dox	1291.8 (M+H)
Example 110	acetyl - P-L-G-(O-(4-pyridyl-)-Y)-Y-L-Dox	1367.6 (M-H)
Example 111	acetyl - P-L-G- (phenylpropyl-G) -Y-L-Dox	1302.4 (M-H)
Example 112	acetyl - P-L-G-(styryl-A)-Y-L-Dox	1300.5 (M-H)
Example 113	acetyl - P-L-G-(O-benzyl-S)-Y-L-Dox	1367.6 (M-H)
Example 114	acetyl - P- (N,N-dimethyl-K)-G-Hof-Y-L-Dox	1333
Example 115	acetyl - P-L-G-Hof-Dap-L-Dox	1213.4 (M+H)
Example 116	acetyl - P-L-G-Hof-Orn-L-Dox	1241.6 (M+H)
Example 117	Peg - P-L-G-Hof-Orn-L-Dox	1359.9 (M+H)
Example 120	acetyl - P-Orn-G-Hof-Orn-L-Dox	1242
Example 121	acetyl - P-Orn-G-Hof-Y-L-Dox	1351

Example 123	acetyl - P-Orn-G-L-Y-L-Dox	1243.3 (M+H)
Example 124	acetyl - P-(4-aza-F)-G-L-Y-L-Dox	1277
Example 125	acetyl - P-L-G-Hof-Dab-L-Dox	1227.6 (M+H)
Example 126	acetyl - P-L-G-Hof-K-L-Dox	1254
Example 127	acetyl - P-L-G-Hof- (N,N-dimethyl-K)-L-Dox	1283.6 (M+H)
Example 129	Peg - P-L-G-Hof- (N,N-dimethyl-K)-L-Dox	1401
Example 132	acetyl - P-L-G-Hof- (N,N-dimethyl-K)-Nle-Dox	1283
Example 133	acetyl - P-L-G-Hof- (N,N-dimethyl-K)-Cha-Dox	1323
Example 134	acetyl - P-L-G-Hof-Cit-L-Dox	1284.4 (M+H)
Example 136	acetyl - P-L-G-Hof-Q-L-Dox	1255.8 (M+H)
Example 137	acetyl - P-L-G-Hof-(4-aza-F)-L-Dox	1275.6 (M+H)
Example 138	acetyl - P-L-G-Hof-V-L-Dox	1224.1 (M-H)
Example 142	acetyl - (homo-P)-L-G-L-Y-L-Dox	1278.578 (M+Na)
Example 143	acetyl - (homo-P)-L-G-Hof-Orn-L-Dox	1256.624 (M+Na)
Example 144	acetyl -Aze-L-G-L-Y-L-Dox	1250.549 (M+Na)
Example 145	acetyl -Aze-L-G-Hof-Orn-L-Dox	1227.585 (M+Na)
Example 146	acetyl -P-L-G-L-Y-G-Dox	1208.5020(M+Na)
Example 147	acetyl -P-L-G-Hof-Y-G-Dox	1256.5040(M+Na)
Example 148	acetyl -P-L-G-L-Y-(β-homo-L)-Dox	1278.5830(M+Na)
Example 149	acetyl -P-L-G-Hof-Y-(β-homo-L)-Dox	1326.5810(M+Na)
Example 150	acetyl -P-L-G-L-Y- (β-Ala)-Dox	1222.5150(M+Na)
Example 151	acetyl -P-L-G-L-Y-Ahx -Dox	1264.5650(M+Na)
Example 152	acetyl -P-L-G-L-Y-Aph -Dox	1326.5820(M+Na)
Example 153	acetyl -P-L-G-L-Y-Amh -Dox	1292.5950(M+Na)
Example 165	acetyl -P-L-G-Hof-(N-methylpiperazinepropyl-G)-L-Dox	1324.6 (M+H)
Example 166	tetrazoleacetyl -P-L-G-Hof-Y-L-Dox	1356.4 (M-H)
Example 167	tetrazoleacetyl -P-L-G-(O-benzyl-S)-Y-L-Dox	1372.5 (M-H)
Example 168	tetrazoleacetyl -P-L-G-Hof-Y-Nle-Dox	1356.5 (M-H)
Example 169	P-L-G-(O-benzyl-S)-Y-L-Dox	1264.5 (M+H)
Example 170	acetyl -P-L-G-Hof-(homoY)-L-Dox	1302.5 (M-H)
Example 171	acetyl -P-AzaHof-G-AzaHof-Y-L-Dox	1340.4 (M+H)
Example 172	acetyl -P-L-G-(O-allyl-S)-Y-L-Dox	1254.6 (M-H)
Example 173	acetyl -P-L-G-(4-nitro-Hof)-Y-L-Dox	1333.4 (M-H)
Example 174	acetyl -P-L-G-Hof-AzaHof-L-Dox	1289.6 (M+H)
Example 175	acetyl -P-L-G-(O-methyl-S)-Y-L-Dox	1228.6 (M-H)
Example 178	3-pyridinecarbonyl -P-L-G-Hof-Y-L-Dox	1353.6 (M+H)
Example 179	2-pyrazinecarbonyl -P-L-G-Hof-Y-L-Dox	1352.7 (M-H)
Example 180	Ac-P-L-G-Hof-K(ME2)-Nle-Dox	1283.5 (M+H)
Example 181	Ac-P-L-G-Hof-Y-Hos Dox	1300.5 (M+Na)
Example 182	Ac-P-L-G-Hof-Y-Hol-Dox	1326.2 (M+Na)
Example 183	Ac-P-L-G-Thr(OBzl)-Y-L-Dox	1342.4 (M+Na)

Table 6e

Example	Cap-P4—P3—P2—P1—P1'—X-Doxorubicin	M/Z:
Example 45	Hyp-G-P-L-G-L-L-Dox	1207
Example 52	acetyl-G-P-L-G-L-L-Dox	1136
Example 53	$O(CH_2CH_2)NCH_2CH_2NHC(=O)$-G-P-L-G-L-L-Dox	1250
Example 54	acetyl-G-P-L-G-L-Y-Dox	1208.5 (M+Na)
Example 56	acetyl-G-P-L-G-Bip-F-Dox	1280
Example 57	acetyl-G-P-L-G-Nle-F-Dox	1170
Example 58	Cbz-G-P-L-G-L-L-Dox	1251
Example 59	$AcHNCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)$-G-P-L-G-L-L-Dox	1306
Example 60	$H_2NCH_2CH_2N(CH_2CH_2)_2NCH_2C(=O)$-G-P-L-G-L-L-Dox	1262
Example 61	Dmg-P-L-G-L-L-Dox	1122
Example 62	acetyl- γ-E -P-L-G-L-L-Dox	1208
Example 63	acetyl-G-P-L-G-Tha-F-Dox	1210
Example 64	acetyl-G-P-L-G-Phg-F-Dox	1190.8 (M+H)
Example 65	methoxyacetyl-G-P-L-G-L-L-Dox	1166
Example 66	Dmg-P-L-G-Tha-L-Dox	1220
Example 67	Dmg-P-L-G-Phg-L-Dox	1199
Example 68	Dmg-P-L-G-(O-benzyl-Y)-L-Dox	1319
Example 69	Dmg-P-L-G-Bip-L-Dox	1289
Example 70	acetyl-G-P-L-G-F-Bip-Dox	1279
Example 71	acetyl-G-P-L-G-L-Bip-Dox	1247
Example 72	acetyl-G-P-L-G-(2Nal)-Bip-Dox	1130
Example 73	acetyl-G-P-L-G-F-A-Dox	1127
Example 74	acetyl-G-P-L-G-Bip-A-Dox	1204
Example 75	acetyl-G-P-L-G-L-A-Dox	1094
Example 76	acetyl-G-P-L-G-(O-benzyl-Y)-F-Dox	1310
Example 77	acetyl-G-P-Q-G-L-L-Dox	1151.8 (M+H)
Example 78	acetyl-G-P-R-G-L-L-Dox	1179
Example 79	acetyl-G-P-L-G-L-(4-pyridyl-A)-Dox	1171
Example 80	acetyl-G-P-L-G-L-R-Dox	1178
Example 81	acetyl-G-P-L-G-L-W-Dox	1208
Example 82	acetyl-G-P-L-G-V-L-Dox	1121
Example 83	acetyl-G-P-L-G-Hof-L-Dox	1184.8 (M+H)
Example 84	acetyl-G-P-L-A-L-L-Dox	1150
Example 85	Dmg-P-I-G-Bip-L-Dox	1232.8 (M+H)
Example 86	Dmg-P-Chg-G-Bip-L-Dox	1258
Example 87	acetyl-G-P-V-G-L-L-Dox	1122
Example 88	Dmg-P-I-G-L-L-Dox	1122
Example 89	Dmg-P-R-G-Bip-L-Dox	1274
Example 90	acetyl-G-P-L-G-L-(O-benzyl-Y)-Dox	1276
Example 91	acetyl-G-P-L-G-E-L-Dox	1152
Example 92	Dmg-P-K-G-Bip-L-Dox	1247
Example 93	acetyl-G-P-L-G-L-E-Dox	1152
Example 94	acetyl-G-P-L-G-Bip-E-Dox	1262
Example 98	acetyl-G-P-L-G-N-L-Dox	1137
Example 99	acetyl-G-P-L-G-S-L-Dox	1110.3 (M+H)
Example 100	acetyl-G-P-L-G-(4-hydroxy-phenyl-G)-L-Dox	1172
Example 140	acetyl-G-Aze-L-G-L-L-Dox	1144.5 (M+Na)

Table 6f

Example	Cap-P4—P3—P2—P1—P1'—P2'—X--Doxorubicin	M/Z:
Example 95	Dmg -P-R-Sar-Hof-R-L-Dox	1384
Example 96	Dmg -P-R-G-Hof-R-L-Dox	1370
Example 97	Dmg -P-R-G-Bip-R-L-Dox	1432
Example 105	acetyl - γ-E -P-L-G-Hof-Y-L-Dox	1419.8 (M+H)
Example 118	acetyl - γ-E -P-L-G-Hof-Orn-L-Dox	1370
Example 119	γ-E -P-L-G-Hof-Orn-L-Dox	1328
Example 122	acetyl - γ-E -P-Orn-G-Hof-E-L-Dox	1386
Example 128	Dmg -P-L-G-Hof-(N,N-dimethyl-K)-L-Dox	1326
Example 130	acetyl - γ-E -P-L-G-Hof-(N,N-dimethyl-K)-L-Dox	1410
Example 131	γ-E -P-L-G-Hof-(N,N-dimethyl-K)-L-Dox	1370
Example 135	acetyl - γ-E -P-L-G-Hof-Cit-L-Dox	1413
Example 139	acetyl - γ-E -P-L-G-Hof-E-L-Dox	1407.4 (M+Na)
Example 156	acetyl -G -P-L-G-L-A-L-Dox	1207
Example 161	Dmg -P-L-G-L-Y-L-Dox	1285
Example 162	Dmg -P-R-G-Phg-Y-L-Dox	1348
Example 163	acetyl -G -P-L-G-L-R-L-Dox	1292
Example 176	acetyl - γ-E -P-L-G-(O-benzyl-S)-Y-L-Dox	1433.5 (M-H)
Example 177	acetyl - γ-E -P-L-G-(O-benzyl-S)-Y-Nle-Dox	1433.5 (M-H)
Example 184	Ac-γ-E-P-L-G-Hof-Y-Nle-Dox	1419.9 (M+H)

Table 6g

Example	Cap-P3—P2—P1—P1'—P2'—P3'--X--Doxorubicin	M/Z:
Example 154	acetyl -P-L-G-L-L-A-L-Dox	1263
Example 155	acetyl -P-L-G-L-Y-A-L-Dox	1313
Example 157	acetyl -P-L-G-L-A-A-L-Dox	1221
Example 158	acetyl -P-L-G-L-A-L-L-Dox	1263
Example 159	acetyl -P-L-G-L-L-S-L-Dox	1279
Example 160	acetyl -P-L-G-L-L-L-L-Dox	1306